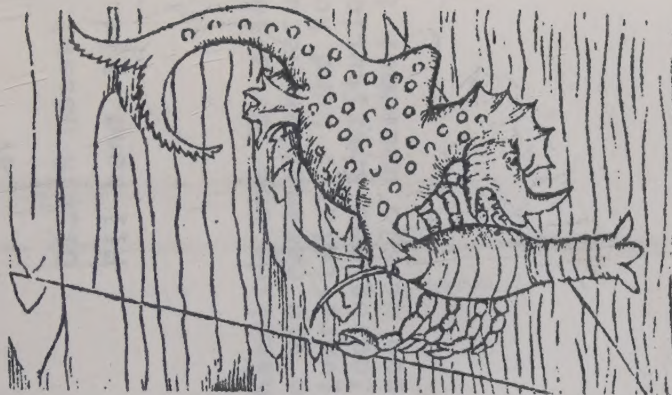


NEURAL CONNECTIVITY PATTERNS
IN
THE COMPOUND EYES OF CRUSTACEANS

by

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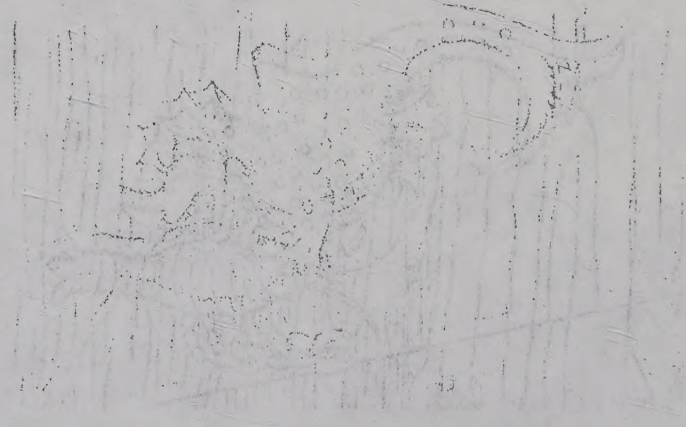


Problems worthy of attack, prove
their worth by fighting back.

Anon.

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The present thesis is a summary and discussion of the following papers:

1. Nässel, D. R.: The organization of the lamina ganglionaris of the prawn Pandalus borealis (Kröyer). Cell Tiss. Res. 163, 445-465 (1975).
2. Nässel, D. R.: The retina and retinal projection on the lamina ganglionaris of the crayfish Pacifastacus leniusculus (Dana). J. Comp. Neurol. 167, 341-360 (1976a).
3. Nässel, D. R.: The fine structure of photoreceptor terminals in the compound eye of Pandalus borealis (Crustacea). Acta Zool. (Stockh.) 57, 153-160 (1976b).
4. Nässel, D. R.: Types and arrangements of neurons in the crayfish optic lamina. Cell Tiss. Res. 179, 45-75 (1977).
5. Nässel, D. R., Waterman, T. H.: Golgi-EM evidence for visual information channeling in the crayfish lamina ganglionaris. Brain Res. 130, 556-563 (1977).
6. Elofsson, R., Nässel, D. R., Myhrberg, H.: A catecholaminergic neuron connecting the first two optic neuro-piles (lamina ganglionaris and medulla externa) of the crayfish Pacifastacus leniusculus. Cell Tiss. Res. 182, 287-297 (1977).
7. Nässel, D. R., Elofsson, R., Odselius, R.: Neural connectivity patterns in the compound eyes of Artemia salina and Daphnia magna (Crustacea: Branchiopoda) (MS, 1977).

The thesis is presented in six chapters reviewing the above papers and papers by other authors. In the five first chapters mainly the crayfish Pacifastacus is discussed, while comparative aspects are discussed in the sixth chapter. The chapters 5 and 6 are to some extent based on previously unpublished results.

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Front illustration: A lobster being
ruthlessly devoured by a "rhinoceros
whale". Wood carving by Olaus Magnus
(1490-1557)

Viele Leute scheinen zu meinen, was man Wissenschaft nenne, sei ganz etwas anderes als das gewöhnliche Wissen, und die Methoden, mit denen man wissenschaftliche Wahrheiten ermittle, verlangten Geistesthätigkeiten von verborgener, geheimnisvoller Natur, welche nur für den Eingeweihten fassbar und hinsichtlich ihres Charakters wie ihres Gegenstandes gleich verschieden seien von dem Verfahren, nach dem wir im gewöhnlichen Leben zwischen Thatsachen und Phantasie unterscheiden.

T. H. HUXLEY (1881)

INTRODUCTION

The visual systems of some insect orders have been extensively studied by numerous workers and the compound eye has proved to be an extremely favourable preparation for vision research (see articles in: Bernhard 1966, Wehner 1972, Horridge 1975, Snyder and Menzel 1975, Zettler and Weiler 1976).

The class Crustacea is taxonomically and phylogenetically well separated from Insecta (see Manton 1973) and comprises mainly aquatic species. Thus, it is of interest to reveal if the compound eyes of the two classes process the visual information in different ways. In order to look for common principles underlying vision in arthropods on the one hand and to detect patterns specific to crustacean (and aquatic) compound eyes on the other, the visual systems of a number of crustaceans have been investigated.

The anatomical features of the crustacean optic lobes (especially the lamina ganglionaris) are discussed in the light of recent investigations on arthropod visual physiology. The present paper is thus an attempt to review the available data on the crustacean compound eye and vision.

1. RETINAL ARRANGEMENTS

(Nässel 1976a)

The anatomy of the retina

The retina of the crayfish compound eye consists of 2000-3000 ommatidia. Each ommatidium comprises a corneal lens formed by two corneagenous cells, a crystalline cone with a long crystalline tract formed by four cells, a rhabdom formed by eight retinular cells and finally two distal pigment cells. In addition, reflecting pigment cells are located proximally near the basement membrane. The number of such cells is not clear but probably one or less than one per ommatidium is present (Parker 1897).

The crayfish retina with its long crystalline cones and stalks is classified as a superposition retina (Exner 1897).

The sensory part, the rhabdom, is formed by microvilli from the eight photoreceptor cells (retinular cells, R1-R8). The main rhabdom is about 150 μm long, spindle shaped, and formed by layers of orthogonally arranged microvilli from seven of the retinular cells (R1-R7) (Rutherford and Horridge 1965, Eguchi 1965). With the numbering of Parker (1897) R1, R2, R5 and R6 form vertically, while R3, R4 and R7 form horizontally arranged microvilli. The nuclei of R1-R7 are located around the distal tip of the rhabdom.

Below the crystalline cone and on top of the R1-R7 rhabdom the R8 cell forms a separate rhabdomere. This small and spindle shaped rhabdomere comprises horizontal microvilli only. The length of the rhabdomere is c. 15-20 μm and largest diameter c. 15 μm .

Below the nucleus R8 forms an axon (diameter c. 2 μm), containing tubules, and mitochondria. This R8 axon accompanies R7 throughout the ommatidium. R8 completely lacks screening pigment granulae.

Neural connectivity patterns in the compound eyes of
Artemia salina and Daphnia magna (Crustacea: Branchiopoda) ¹⁾

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INTRODUCTION

In order to appreciate basic neural principles in arthropod vision it is valuable to compare the visual systems of animals with different degrees of complexity. The neuronal connectivity pattern is relatively well known in specialized arthropod compound eyes. This refers in crustaceans to the order Decapoda and to the first optic neuropil region, the lamina ganglionaris (Hafner 1973, Nässel 1975, 1977, Nässel and Waterman 1977, Stowe et al. 1977).

The crustacean cohort Branchiopoda comprises animals presumed to be less specialized and whose eyes deviate markedly from those of decapods and most other malacostracan crustaceans (Debaisieux 1944, Röhlich and Törö 1965, Güldner and Wolff 1970, Elofsson and Odselius 1975).

The neuronal topography and characteristics of the optic neuropils of these animals are, however, poorly understood. The only animal who has received some attention in this respect is Daphnia (Retzius 1906, Leder 1915, Hanström 1928, Wolff and Güldner 1970, Macagno et al. 1973, Macagno and Levinthal 1975).

The present investigation performed on the easily accessible animals Artemia salina and Daphnia magna is based on Golgi preparations and EM. It attempts to classify the neuron types and discuss their connectivity patterns and create a basis for comparison between compound eyes of different degrees of complexity.

MATERIAL AND METHODS

The animals used in this investigation are Artemia salina L. reared from commercially available eggs (see Elofsson and Odselius 1975), and Daphnia magna, Straus, obtained from local ponds.

For light microscopy selective silver methods were used. The Golgi-Colonnier method as adapted by Strausfeld and Blest (1970)

(see also Nässel 1977) was successfully used. The osmic acid prefixed Golgi-Colonnier (Nässel 1977) gave more selective and clearer impregnations on Artemia. The Golgi rapid and Golgi-Cox methods were tried but gave no results.

To obtain good Golgi preparations of Artemia the head had to be cut in two halves and then immersed in fixative according to Karnovsky (1965) for three hours. Attempts to stain single eyestalks or even dissected optic lobes failed. For Daphnia the heads were cut off and immersed in fixative.

The minute optic lobes of these animals required maximum dichromate impregnation and silver treatments of 3-5 days each. The impregnated optic lobes were dehydrated and embedded in Spurr's medium (Spurr 1969). 30 μm sections of Artemia and 60 μm of Daphnia were cut on a sliding microtome and mounted under coverslips with Permount.

Drawings of neurons were made using a Leitz drawing tube on a Leitz Orthoplan microscope equipped with a x100 oil immersion planapochromate objective. Photographs were taken on the same microscope with a Leitz automatic camera.

For electron microscopy eyestalks of Artemia and Daphnia were fixed in glutaraldehyde-paraformaldehyde according to Karnovsky (1965) in cacodylate buffer (pH: 7.3) for three hours. After a thorough wash in buffer the tissue was fixed in 4% OsO_4 (in the same buffer) for two hours. Dehydration was performed in ethanol and the tissue was embedded in Vestopal W. Block staining was performed in 1% phosphotungstic acid and 0.5% Uranyl acetate. Sections were cut on an LKB Ultratome, and examined in a Zeiss EM 10 electron microscope and a Philips EM 300.

RESULTS

The optic lobes of Artemia salina

General organization

The stalked eyes of Artemia are small (length of the eye stalk c. 0.5-1.0 μm). The apposition type retina is half-sphere shaped and consists of c. 300 ommatidia. In the eyestalks two neuropil regions are found central to the retina: the lamina ganglionaris and the medulla (medulla externa). The remaining optic neuropil regions are withdrawn into the protocerebrum. Between the lamina and the medulla no chiasma is formed as seen in malacostracan crustaceans, but straight axon bundles connect the two neuropil regions.

Cellbodies (mainly neural cellbodies) reside on the distal lamina border and on the lateral medulla periphery (Fig. 6).

Retina-lamina projection

The ommatidia each consists of six retinular cells which form rhabdomeres (Elofsson and Odselius 1975). One of these is aberrant. Distally in the ommatidium six rhabdomeres are present forming a rhabdom with four microvilli directions. Upon withdrawal of the R6 rhabdomere its space is filled by R1 and only five rhabdomeres constitute the rhabdom which still has four microvilli directions.

The receptor axons penetrate the basement membrane without any specific pattern. In the passage the axons become thinner and unpigmented. No interweaving of axons is found in this region as described in decapods (Kunze 1967, Meyer-Rochow 1975, Nässel 1976a), but the axons from each ommatidium join in bundles of six just below the basement membrane. Slightly deeper the axons are densely packed together so that individual groups of six cannot be resolved.

Further down the axon diameters become smaller and they are unpigmented. In this region groups of six photoreceptor axons

reappear (Fig. 1b). As no serial sectioning has been performed over this long distance we do not know if these groups are identical to the groups below the basement membrane (i.e. if each group of six axons represents one ommatidium).

When entering the cellbody layer of the lamina, axons from monopolar neurons join the photoreceptor axon bundles. Thus, "pseudocartridges" are formed which just above the lamina plexiform layer each consists of six photoreceptor axons and four to five other axons (Figs 1a, c). This periodicity is maintained in the lamina neuropil so that axons of the pseudocartridges form synaptic compartments (see below).

Types of neurons in the lamina

In the lamina of Artemia one can distinguish five classes of neurons: photoreceptor axons, monopolar neurons, centrifugal neurons, tangential neurons, and amacrine (anaxonal) neurons. The following descriptions are based on Golgi preparations.

Photoreceptor axons. Three types of photoreceptor axons can be distinguished (RT_1 , RT_2 , and RL) (Figs 4, 10). Two of these types have terminals in the lamina (RT_1 and RT_2) and one (RL) has a long axon ending in the medulla.

RT_1 are characterized by enlarged, branched terminals in the distal part of the lamina plexiform layer. From the enlarged part of the terminal numerous thin, long processes spread radially over a diameter of 35-55 μ m. The terminal portion of this axon extends to about half the plexiform layer depth.

RT_2 axons have long, thin, blebbed terminal trunks with radially arranged thin processes. The trunk with its processes occupies the lower half of the plexiform layer. With the thin lateral processes the terminals have diameters of 30-75 μ m.

There is some overlap between RT_1 and RT_2 terminal processes in the longitudinal axis so that no clearcut border between the two terminal strata can be observed.

Laterally the receptor terminals overlap considerably by means of the long lateral processes.

RL, the long visual fibre, is thin and can be traced to the proximal part of the medulla. A terminal has been disclosed shallowly in the medulla (Fig. 6). In the lamina some thin processes emerge from the RL axon.

Monopolar neurons. The variation in the morphology of lamina monopolar neurons is extensive. When classifying the neuron types it is therefore difficult to obtain sharp divisions between the types in light microscopy only. As only one cell-body layer distal to the lamina is present in Artemia, a classification based on site of cellbodies cannot be made as for decapod crustaceans (Nässel 1975, 1977).

The criteria used here for dividing the monopolar neurons into relevant groups are: stratification of the lamina lateral processes, and number and lateral width of these processes. With these criteria four types of lamina monopolar neurons can be resolved (M_1 - M_4) (Fig. 4).

M_1 has an axon that in the lamina has very few and short or no processes. The lateral processes can be found at any level in the plexiform layer. The cellbodies are located just distal to the plexiform layer resulting in very short cellbody fibres in M_1 . It is, however, possible that M_1 neurons actually are M_2 neurons (see below) that are only fragmentary stained.

M_2 neurons have radially arranged branches throughout the lamina plexiform layer. The lateral width and number of these processes vary. The average diameter of M_2 including its processes is 18 μ m. The cellbodies do not have constant positions so that the cellbody fibres can vary in length from very long to short as in M_1 .

M_3 and M_4 are stratified monopolar neurons. M_3 has lateral processes in the distal and M_4 in the proximal part of the

plexiform layer. On basis of these two stratified neurons and the receptor terminals in two corresponding levels the lamina plexiform layer can be divided into two sublayers, ep1₁ and ep1₂. These layers are, however, not easily resolved in light microscopy since the Artemia lamina is small and exact orientations of the sectioning planes are difficult to obtain.

Each of the neurons (M_1 - M_4) has lateral processes that appear to be restricted to one synaptic compartment in the lamina only (though the long processes of the receptor terminals may contact monopolar neurons in several such compartments). No wide field monopolar neurons are resolved. There is an extensive variation in the length of the lateral processes of the M_1 - M_4 neurons. This variation is most likely due to the location of the individual neurons in the synaptic compartments and the size of these compartments (see discussion).

Tangential neurons (Figs 5, 6). Large neurons of T-neuron type have tangentially arranged processes in the lamina plexiform layer. These processes have a large lateral extent (c.125 μ m), sometimes covering most of the lamina in horizontal sections. Secondary and tertiary processes penetrate the whole lamina depth, sometimes in a bistratified manner. These neurons have been designated Tan₁.

A thick axon connects the lamina part with deeper optic neuropil regions in protocerebrum. This axon runs outside the medulla, but the cellbody fibre passes through the medulla. The large cellbody is found in the medulla cellbody layer. No processes or other specializations can be seen on the cellbody fibres in the medulla indicating that no synaptic connections are present in the medulla for this neuron.

Up to four neurons of this type have been found in the same sections several times. Their lateral branches overlap conspicuously. Totally the lamina appears to contain about 8-12 such neurons.

Centrifugal neurons (Figs 5, 6). Another type of neuron (C_1) is found with tangential processes in the lamina. This type is, however, not of T-neuron type, but is monopolar with its cellbody in the medulla cellbody layer (MCL). From the cellbody an axon penetrates the medulla, where it has lateral processes in a stratum close to the MCL. The axon then passes to the lamina where tangential branches are spreading c.70 μ m laterally. The arborization pattern resembles that of the tangential T-neurons, but their lateral extent is somewhat smaller and the main axon diameter is smaller. Neurons with this basic morphology are in insects referred to as centrifugal neurons (Trujillo-Cenoz and Melamed 1970).

Amacrine neurons (Fig. 6). Cells are distinguished with cellbodies distal to the lamina and processes profusely branching in the plexiform layer. The lateral extent of the lamina arborization is c.25 μ m. The processes can emerge from one single short cellbody fibre or occasionally in a multipolar pattern with several processes from the cellbody. No axon is seen that connects the lamina with the medulla (or other parts of the lamina). These cells can therefore be classified as anaxonal or amacrine neurons (Am).

Types of neurons in medulla externa (Fig. 6)

The medulla contains the terminals of the RL and M_1 - M_4 axons. Only the M_2 and RL terminals have been resolved with certainty since the medulla is a quite complicated neuropil region and mass stainings often obscure the individual neurons. The M_2 axon ends shallowly in the medulla neuropil with a diffusely branching terminal. RL has a shallow terminal with a few lateral processes.

Another neuron type that is shared by the lamina and medulla is the centrifugal neuron C_1 described above. In the medulla C_1 s have lateral processes forming a plexus close to the medulla cellbody layer. These processes overlap each other to some extent.

Centripetal monopolar neurons connect the medulla with protocerebrum. By analogy with previous studies of insect optic lobes (Strausfeld and Blest 1970) these centripetal neurons are termed transmedullary neurons (TM). The TM neurons (of which two classes have been distinguished so far) have their cellbodies in the MCL. Their axons penetrate the medulla where they have lateral branches in the same plexus as the C_1 neurons. The axons then pass via the "optic nerve" to protocerebrum. The two classes of TM neurons differ in the medulla arborization pattern. TM_1 has a smaller and TM_2 a larger field of arborization.

Neurons with their cellbodies in protocerebrum connect this region with medulla. Their medulla branches extend over a large part of the neuropils.

Finally, there are amacrine neurons in the medulla with cellbodies in the medulla cellbody layer, and diffusely branching processes in the medulla neuropil.

Fine structure of the lamina

The pericarya of the monopolar cells are rich in mitochondria and Golgi complexes and at the axon hillocks tubuli appear. Only one morphological type of pericaryon was found. Their axons one by one join the bundles of photoreceptor axons. Distal to the plexiform layer bundles each consisting of 10-11 axons are found (so called pseudocartridges) (Fig. 1c). Here it is difficult to distinguish the photoreceptor axons from the monopolar axons. Both types contain tubuli and mitochondria.

Some of the photoreceptor axons in each pseudocartridge gradually become enlarged in the distal lamina neuropil and the monopolar axons form branches. Thus, a periodic organization can still be recognized in the distal part of the lamina neuropil (Figs 1d, e).

The impression is that in this region each of the periodic groups contains two enlarged receptor terminals, the remaining

four receptor axons and the branching monopolars.

The periodic organization gradually disappears deeper in the lamina neuropil (i.e. it is more difficult to resolve) (Figs 1e, 11). That the periodic organization is difficult to recognize is easily explained by the extensive arborizations of receptor terminals. In addition, there is a high number of tangential processes in the distal and proximal parts of the plexiform layer further confusing the picture.

The photoreceptor terminals contain large mitochondria, some tubules, but are otherwise electron lucent. The terminals lack rodshaped inclusions (filament fascicles) and have a low concentration of clear vesicles, thus differing from decapods (Hafner 1973, Nässel 1976a, b). The clear vesicles in Artemia are concentrated around synaptic sites.

The receptor axons form three types of specialized contacts with other elements. The first type (Figs 2c, f) is characterized by a small terminal evagination containing a dense bar and a cluster of clear vesicles (diameter of vesicles 30 nm). In analogy with previous studies this contact can be considered as a synapse. There are always two postsynaptic profiles at this type of junction (dyad synapse). The postsynaptic profiles are not yet identified, but most likely at least one of the elements is a spine from a monopolar neuron. No invaginating spines from the postsynaptic profiles into the terminals have been identified as are seen in the crayfish (Nässel and Waterman 1977).

The second type of specialized contacts are formed between the trunks of photoreceptor terminals (Figs 2a, b). The contacts are formed by means of finger like protrusions from one terminal into another. These protrusions have a diameter of c. 0.3 μ m. Clear vesicles are clustered on one or both sides of the membrane contacts. All the morphological criteria of a true synapse are not fulfilled by this type of contact.

A third type of synapse can be seen between RT's and unidentified processes (Fig. 2h). This third type of synapse is an

assymetrical one with a density and a cluster of vesicles at the presynaptic membrane. Only one postsynaptic profile is present in this type. There is a densification of the postsynaptic membrane.

Occasionally RT's have been found to be postsynaptic to unidentified processes (Fig. 2g). The RT profiles are then one of the two profiles in a dyad (this is a second type of dyad distinct from the one described above).

Between the identifiable RT-trunks numerous smaller profiles are seen. Due to the repeated arborizations of the axons, the locations of the monopolar neurons are impossible to resolve.

Long, thin, tangentially oriented fibres form dense networks around the periodic elements. Two morphological types are distinguished. One type is recognized by its contents of tangentially arranged microtubuli and mitochondria only; the other by having in addition small dense cored vesicles (Figs 2d, e).

The small dense cored vesicles have a diameter of 70-80 nm. Also clear vesicles are present in these processes. These fibres are occasionally found presynaptic to RT's (Fig. 2i). Both types of tangential profiles have diameters of c.0.5µm. No profiles are found with large dense cored vesicles or vesicles of neurosecretory type (c.p. Pacifastacus; Elofsson et al. 1977).

Marginally, just distal to the lamina plexiform layer glial cells are situated (Fig. 1a). These cells have long lobulated nuclei and send long, thin processes enwrapping the pseudo-cartridges. Their cytoplasm is electron dense and contains rough endoplasmic reticulum, free ribosomes, elongated mitochondria and microtubuli. The glial processes appear to penetrate into the lamina plexiform layer where they separate laminar elements at some levels.

Daphnia magna

General organization

The globular compound eye of Daphnia is composed of originally paired eyes and this fusion is extended to encompass also the lamina ganglionaris. The medullae externae, however, are still separated. The ommatidia contain eight reticular cells whose axons continue as separate bundles to the lamina. Entering the lamina five laminar neurons adhere to each bundle (Macagno et al. 1973).

Types of neurons in the lamina

The neurons in the visual pathway have been investigated with the Golgi technique (Retzius 1906), vital dyes (Leder 1915) and computer aided serial reconstruction from electron micrographs (Macagno et al. 1973). We therefore have a comparatively good knowledge of the neuron types although our results show that more information still can be added. The lamina contains photoreceptor terminals, monopolar neurons, centrifugal neurons, tangential neurons and amacrine cells.

Photoreceptor terminals. Retzius (1906) and Macagno et al. (1973) portrayed only one type of receptor terminal ending with a comparatively small dilatation having an array of widely distributed branches. Leder (1915) showed no such dilatations which may be due to the technique used.

The present investigation has revealed two morphological types of receptor terminals (Figs 7, 8); one having a flat, short dilatation either distally (RT_1) or proximally (RT_3) in the lamina and another having a longer dilatation in the middle part of the lamina (RT_2). All types branch profusely. The branches have a large circumferential extension and occupy in the case of RT_2 approximately 1/4 of the horizontal extension of the lamina whereas RT_1 and RT_3 occupy approximately 1/3. Absolute values of the extensions are dependent on the size of the animals and can vary between 25 and 100 μm . The lamina "diameter" varies between 100 and 200 μm . Only the

marginal RT's have a restriction in the spread of the branches, all other having an equal distribution around the axon. There is no limit for RT-branches imposed by the midline between the two halves of the lamina. So far no receptor terminals have been found to bypass the lamina and end in the medulla and this is in agreement with previous investigations, except Macagno and Levinthal (1975) who claim that two photoreceptor axons terminate in the medulla. A certain variation of the branching pattern occurs within each type of terminal (Fig. 10). This has been shown by Macagno et al. (1973) and can be confirmed here. The variation takes place within individuals and between species. Thus, sometimes RT₂'s are found which have very wide branches almost half the width of the lamina. Among the RT₃ type many has none, but some neurons have spines along the axon distal to the dilatation. When a rich branching occurs, this could be a RT₂ less dilated than normally.

When all RT's occur together in a section of the lamina (Fig. 8), a stratification is disclosed which is seen as a thin distal layer of RT₁, a broad middle layer of RT₂, and finally a thin proximal layer of RT₃ terminals. The branches, however, do overlap and thus no sharp borders can be distinguished.

With regard to the somewhat indistinct classification of RT₂ and RT₃ terminals, the possibility cannot be ruled out that there are only two strata: one consisting of RT₁ and one of the RT₂ and RT₃ axons, which then should be variations of one type (see Fig. 10).

Monopolar neurons (Fig. 7). Retzius (1906) showed a bistratified monopolar neuron, which is confirmed here, and which could correspond to the terminal layers of RT₁ and RT₃. In addition, we have found one monopolar type branching richly in a broad part of the middle stratum of the lamina. This latter corresponds to the RT₂ extension in the lamina. The branching axon portion is also somewhat expanded. The width of this Daphnia monopolar neuron is somewhat less than the terminals in the same stratum.

Both types of monopolars described so far have a wide and rich branching in the medulla.

Macagno and Levinthal (1975) described one monopolar neuron with a synaptic region in the posterior neuropil region and three with a bistratified pattern. No figures are given but they could be interpreted as similar to the types above.

Tangential neurons (Fig. 9). Retzius (1906) and Leder (1915) showed two types of connections between the lamina and the brain; one connects both the lamina and the medulla with the brain; the other is a direct route between the lamina and the brain (Fig. 9). The cellbodies of these two types are situated in the medulla cellbody layer.

Retzius and Leder also figured neurons connecting the lamina and the brain but with cellbodies either in the brain or in the lamina. These types have not been verified by us.

The neuron connecting both the lamina and the medulla with the brain is peculiar to Daphnia and has a very wide and loose (airy) distribution in the optic neuropils. It ends in the ipsilateral side of the brain in the portion equivalent to the medulla terminalis.

Centrifugal neurons (Fig. 7). Monopolar neurons are present whose cellbody is situated in the medulla cellbody layer. Their axons branch in the medulla and continue to the lamina where they branch again. The branches are rather restricted in extent and appear in the impregnations as thick and grape-like clusters. The lamina branches occur in the middle part of the lamina plexiform layer. According to Retzius and Leder, this connection is made by T-neurons.

Amacrine neurons (Fig. 7). Neurons restricted to the lamina have been described by Retzius (1906), Leder (1915) and Macagno and Levinthal (1975). They are also named amacrine cells and can be either multipolar or have a short cellbody

fibre giving rise to a richly branching tree. Their cell-bodies are often found laterally and the branches cover most of the lamina.

Types of neurons in the medulla externa

Apart from the neurons described above and shared with the lamina, the medulla has in addition so called transmedullary neurons. These branch profusely in the medulla, have their cellbodies in the medulla cellbody layer and connect with the medulla terminalis part of the brain.

One such neuron is figured by Retzius (1906) who also showed a centrifugal neuron from the brain. The connection between the medulla and the brain is exclusively of the latter type according to Leder (1915) and Macagno and Levinthal (1975).

Fine structure of the lamina

The entrance of the eight-axon bundles from the retina in the lamina can be followed easily through the cellbody layer as is seen also in the light microscope. In this region the axon bundles receive additional fibres from the lamina neurons. The glial investment is prominent in this region and the electron lucent thick glial sheaths separate the pseudo-cartridges (Fig. 3a). Entering the neuropil proper this orderly arrangement is lost. The glial investment is reduced to very thin lamellae and the extensive branching and terminal dilatations conceal any pattern (Fig. 3b). Lamina neurons and reticular cell axons appear very similar in the sections and offer thus no support in differentiating between the two fibre types and their branches. Recognizable structures are the dilated reticular cell axon terminals, although it must be kept in mind that the monopolar neuron type described here also is dilated. Further thin spine-less fibre profiles, usually in pairs or three together, are repeatedly found. These could be either the bare portions of the lamina monopolar neurons or the naked distal part of the terminals of type RT₃. The neuropil organization is thus even less structured than in Artemia.

The intricate pattern of fibres in the lamina plexiform layer precludes an intelligible description of connectivity between the neuron types found in ordinary electron micrographs. With the serial section reconstruction technique, Macagno and Levinthal (1973) have established synapses between receptor terminals themselves and between receptor terminals and lamina neurons. In the latter case four receptor neurons were found to have one main receiving lamina neuron although each receptor terminal could make synapses onto two different lamina neurons. The synapses were classified as being of the classic parallel type. Wolff and Güldner (1970), however, distinguished three different morphological types: one characterized by a dome-shaped protrusion from the presynaptic into the postsynaptic fibre, one being of the "normal" parallel type and one containing a dense bar surrounded by clear vesicles. These types have been found also in the present investigation (Figs 3c, d). The validity of these types has not been established by serial sectioning. Thus, we cannot rule out the possibility that they are all one type sectioned in different planes (Figs 3c, d).

Wolff and Güldner (1970) also described fibre profiles containing dense cored vesicles and such with dense vesicles of "neurosecretory" type. This can be verified here. Fibres containing dense cored vesicles can be suspected to contain a biogenic amine and it can be noted that with the histochemical fluorescence method of Falck and Hillarp a green fluorescence was demonstrated in the daphnid lamina and medulla suggesting catecholaminergic neurons to be present (Aramant and Elofsson 1976).

DISCUSSION

The organization of the compound eyes of Daphnia and Artemia is clearly less complicated than that of decapod crustaceans both at the retinal and neuropil levels. The retina has been discussed elsewhere (Röhlich and Törö 1965, Güldner and Wolff 1970, Elofsson and Odselius 1975) so we will now focus on the optic lobes.

Before discussing details of neural organization in Daphnia and Artemia some differences and similarities between the species can be summarized as follows.

Features that are shared by Daphnia and Artemia but are not found in decapods are:

1. The extensively branching photoreceptor terminals.
2. The lack of columnar organization of the lamina plexiform layer.
3. The presence of tangential neurons connecting the lamina directly with protocerebrum.

Daphnia differs from Artemia in the following ways:

1. Possibly the layering of RT's in Daphnia differs from the bistratified pattern in Artemia.
2. In Daphnia no long reticular cell axons are found so far.
3. No dyadsynapses are found in Daphnia.
4. Neurosecretory fibres occurring in Daphnia are absent in Artemia.
5. No wide field centrifugal neurons of Artemia type are resolved in Daphnia.
6. The presence of tangential neurons connecting both the lamina and the medulla with protocerebrum in Daphnia.
7. Daphnia monopolar neurons of only two types are found so far (c.p. 4 types in Artemia).

Artemia optic lobes

Although a periodic organization can be seen distally in the lamina neuropil, no clear compartments of neural elements are resolved. The retinotopically projecting receptor axons are collected together with monopolar axons in pseudocartridges that invade the lamina neuropil, where they most likely form functional units. The extensive branching of photoreceptor terminals and other neurons and the lack of neuroglial ensheathening of compartments make these units difficult to distinguish.

In several insects (Strausfeld 1976) and decapod crustaceans (Hafner 1973, Nässel 1975, 1976a, b) synaptic units termed cartridges can be identified. These are more or less separated from each other by neuroglial cells. The Artemia pattern with no clear cartridges might represent a more simple level of organization.

The photoreceptor terminals obviously interact laterally with other elements by means of their numerous long processes. With conventional EM it has not been possible to identify the connectivity patterns of these terminal processes. Possibly these long processes synapse on elements in adjacent synaptic units and form circuits for lateral inhibition. This is an interesting arrangement since normally the receptor terminals have a very restricted domain and instead second order neurons mediate lateral contacts. Apart from Daphnia and Artemia described here, this arrangement has also been seen in the lamina of the hemipterous bug Notonecta (Strausfeld 1976).

The invaginations from one receptor terminal into another with clear vesicles along the membrane contacts might represent synaptic contacts between receptors. This type of contacts has been seen between receptors of the same synaptic unit only. The most likely function of such contacts would be coupling between receptor terminals resulting in signal amplification. Chi and Carlson (1976a, b) demonstrated similar contacts between receptor terminals in the fly Musca.

The long receptor axons (RL) possibly belong to the small R6 cells, by analogy with long receptor axons in Pacifastacus belonging to the small R8 (Nässel 1976a).

The dyad synapses formed by the receptor terminals infer output on two second order neurons instead of three found in decapods (Nässel and Waterman 1977). In decapods, all identified synapses where RT's are presynaptic are triads (Hafner 1974, Nässel 1976a, Nässel and Waterman 1977). In the crayfish the profiles of the triads are identified. In all epl₁ triads the profiles are M1, M2 and M3, and in epl₂ triads they are M1, M2 and M4. It is of particular interest to unravel which element that is missing in the Artemia synapse complexes.

By analogy with the crayfish the two elements of the dyads might be M2 and M3, and M2 and M4 respectively in the two strata. Then the third crayfish element, M1, should be the one missing in Artemia.

The large Tan 1 neurons are interesting since they connect the lamina directly with the protocerebrum and thus relay less integrated information to protocerebral centres. This type of neurons has not been identified in decapods. Zawarzin (1914), however, demonstrated a similar neuron type in Aeschna larvae connecting the lamina with deeper optic neuropil regions. The Tan 1 neurons resemble neurons connecting medulla externa with protocerebrum in decapods (Nässel unpublished). Tan 1 neurons cover a large lamina portion and might represent motion sensitive units or units relaying input on optomotor neurons.

The wide field centrifugal monopolar neurons (C 1) are so far unique to Artemia among crustaceans. Neurons with long tangential lamina processes are all T-neurons in decapods. C 1 is the only type of wide field neuron connecting the lamina with the medulla. Since C 1 appears to be centrifugal in nature, there are no neurons distinguished in Artemia that directly can transfer signals from a larger portion of the retinal mosaic from the lamina to the medulla as seems possible in decapods (Nässel 1975, 1977, Stowe et al. 1977).

The final major difference between Artemia and decapods is the lack of profiles with large dense vesicles. In Pacifastacus two types of profiles are distinguished with dense vesicles. One with smaller vesicles probably represents catecholaminergic neurons, the other with larger vesicles might be neurosecretory (Elofsson et al. 1977). There are also specialized contacts between these two fibre types in Pacifastacus (Nässel unpublished). In Artemia the vesicles appear to interact with visual interneurons and possibly receptor terminals. Aramant and Elofsson (1976) demonstrated green fluorescence with the Falck-Hillarp technique in the Artemia

lamina indicating a catecholamine. Thus, the profiles with dense cored vesicles might represent catecholaminergic neurons. The fluorescence pattern is not possible to match with any of the neuron types resolved in the Golgi technique.

The medulla externa has not been described in detail for any crustacean except Daphnia (Retzius 1909, Leder 1914). Inspections of some decapod medullae (Nässel unpublished) together with Hanströms (1928) schematic diagrams can be compared with the present description of the Artemia medulla. It is clear that the Artemia medulla is less complex (the medulla interna is completely lacking). It is relatively smaller, contains fewer neuron types, and has fewer strata. There is thus a lower degree of peripheral integration in the Artemia visual system.

Daphnia optic lobes

The stratification of the lamina caused by the different endpoints of the receptor terminals in three layers deviates both from Artemia and decapods. The extent to which this is a division also into functional layers or merely a mechanical way of distributing numerous large terminals is not known. In the former case it would require a counterparting branching pattern of e.g. monopolar and tangential neurons of the lamina. There are no unistratified monopolar neurons revealed that are clearly corresponding to either of the RT strata. The bi-stratified monopolar neurons though indicate that at least two layers are present: one distally and one proximally in the plexiform layer. In the case that RT2 and RT3 terminals are variations of one type, only two layers exist and they are then similar to those of Artemia.

The lack of long visual fibres in our Golgi preparations and those of Retzius (1906) and the methylene blue preparations of Leder (1915) might only be due to failure to stain such cells. If all reticular cells will prove to end in the lamina it could be correlated with the fact that no aberrant reticular cell is found in the ommatidia of Daphnia as e.g. the

eighth cell in many decapods or the sixth cell in Artemia ommatidia. Macagno and Levinthal (1975) claimed that two receptor axons per ommatidium bypassed the lamina and had terminals in the medulla. They, however, gave no clear documentation of this.

Since four monopolars per "cartridge" have been demonstrated (Macagno et al. 1973, Macagno and Levinthal 1975) that have different connections in the lamina, it is probable that some morphological types of monopolar neurons have been resistant to staining. There might thus be more types than the bistratified monopolar found by Retzius (1906) and in this investigation and the unstratified one described by us.

The presence of one to one synapses might infer receptor output on just one second order (monopolar) neuron. But it is rather probable that second order neurons contact the RT's at different sites (Macagno and Levinthal 1975) and that there are thus more than one postsynaptic neuron per RT.

Conclusion

Although the retinal and neuropil organization of Artemia and Daphnia is less complicated than that of e.g. decapod crustaceans, these animals are capable of a relatively good visual performance (Seifert 1932, Waterman 1966, Young 1974, Frost 1975, Young and Downing 1976). This is supported by the present findings of a relatively varied and well developed population of visual neurons.

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FIGURE LEGENDS

Fig. 1 The lamina of Artemia.

- Fig. 1a Pseudocartridges distal to the lamina. A large neuroglial cell with a lobulated nucleus (NG) form sheaths (arrows) around the pseudocartridges. The elements of one pseudocartridge are labeled: large asterisks mark monopolar axons (more axons join deeper down); small mark receptor axons. NM = nucleus of monopolar neuron.
Magnification: x 8060
- Fig. 1b Three bundles of six photoreceptor axons distal to the lamina cellbody layer.
Magnification: x 9 875
- Fig. 1c Pseudocartridges just distal to the lamina plexiform layer. One pseudocartridge with 11 axons is ensheathed by glial processes (arrow heads). MA = axon hillock region of monopolar neuron. NM = nucleus of monopolar neuron in adjacent pseudocartridge.
Magnification: x 15 590
- Fig. 1d Pseudocartridge just entering epl_1 . The axons have started to expand and branch. Two receptor terminals (asterisks) are enlarged. G = neuroglial process.
Magnification: x 9 400
- Fig. 1e Cross section of the lamina plexiform layer (deep in epl_1). A periodic arrangement can barely be resolved. Each asterisk marks one periodic group of nervous elements. R = receptor axon terminal.
Magnification: x 6 457
- ### Fig. 2 The lamina of Artemia.

- Fig. 2a Finger-like protrusions (asterisks) connecting different RT's by invagination. Note clear vesicles along the membrane contacts.
Magnification: x 33 820
- Fig. 2b RT protrusion (from Fig. 2a) in higher magnification. With the goniometer no membrane specializations could be detected.
Magnification: x 58 800
- Fig. 2c Three dyad synapses (arrow heads). An RT is the pre-synaptic profile.
Magnification: x 58 800
- Fig. 2d Tangential lamina process (dc) with small dense cored vesicles.
Magnification: x 12 860
- Fig. 2e Dense cored vesicles in lamina fibres.
Magnification: x 58 800
- Fig. 2f-i Synapse types in the lamina of Artemia. RT = photoreceptor axon terminals.
- Fig. 2f Dyad synapse. Note dense presynaptic bar and clear vesicles.
Magnification: x 79 375
- Fig. 2g Dyad synapse with an unidentified presynaptic profile (asterisk) and an RT as one of the postsynaptic elements.
Magnification: x 58 800
- Fig. 2h Synapse type with only one postsynaptic profile. Note dense bar, clear vesicles and dense postsynaptic membrane.
Magnification: x 79 375

Fig. 2i A fibre profile (dc) with dense cored vesicles is presynaptic to an RT by means of a "parallel" synapse. Note clear vesicles and dense pre- and postsynaptic membranes.

Magnification: x 58 800

Fig. 3 The lamina of Daphnia.

Fig. 3a Pseudocartridges distal to the lamina. Two such units are shown separated by means of neuroglial cells with clear cytoplasm. The arrow points at the border between two neuroglial cells. Each pseudocartridge contains five monopolar axons (asterisks) (note that one of these might be an amacrine neuron), and eight photoreceptor axons (small rings).

Magnification: x 7 500

Fig. 3b Cross section of lamina plexiform layer. No clear periodic organization can be disclosed.

Magnification: x 10 220

Fig. 3c Synapse between two lamina elements (arrow). A dense bar and clear vesicles characterize this type. Magnification: x 106 750.

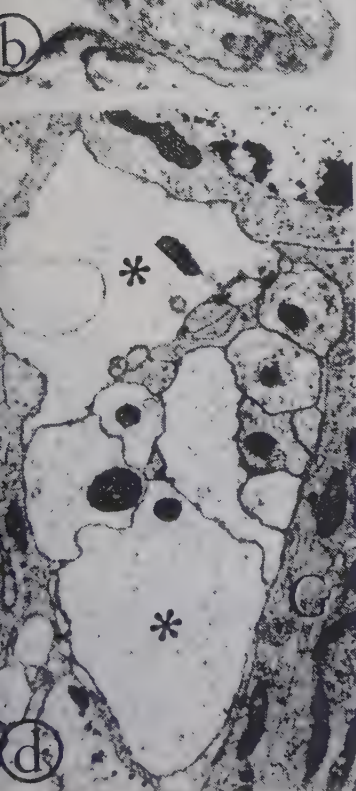
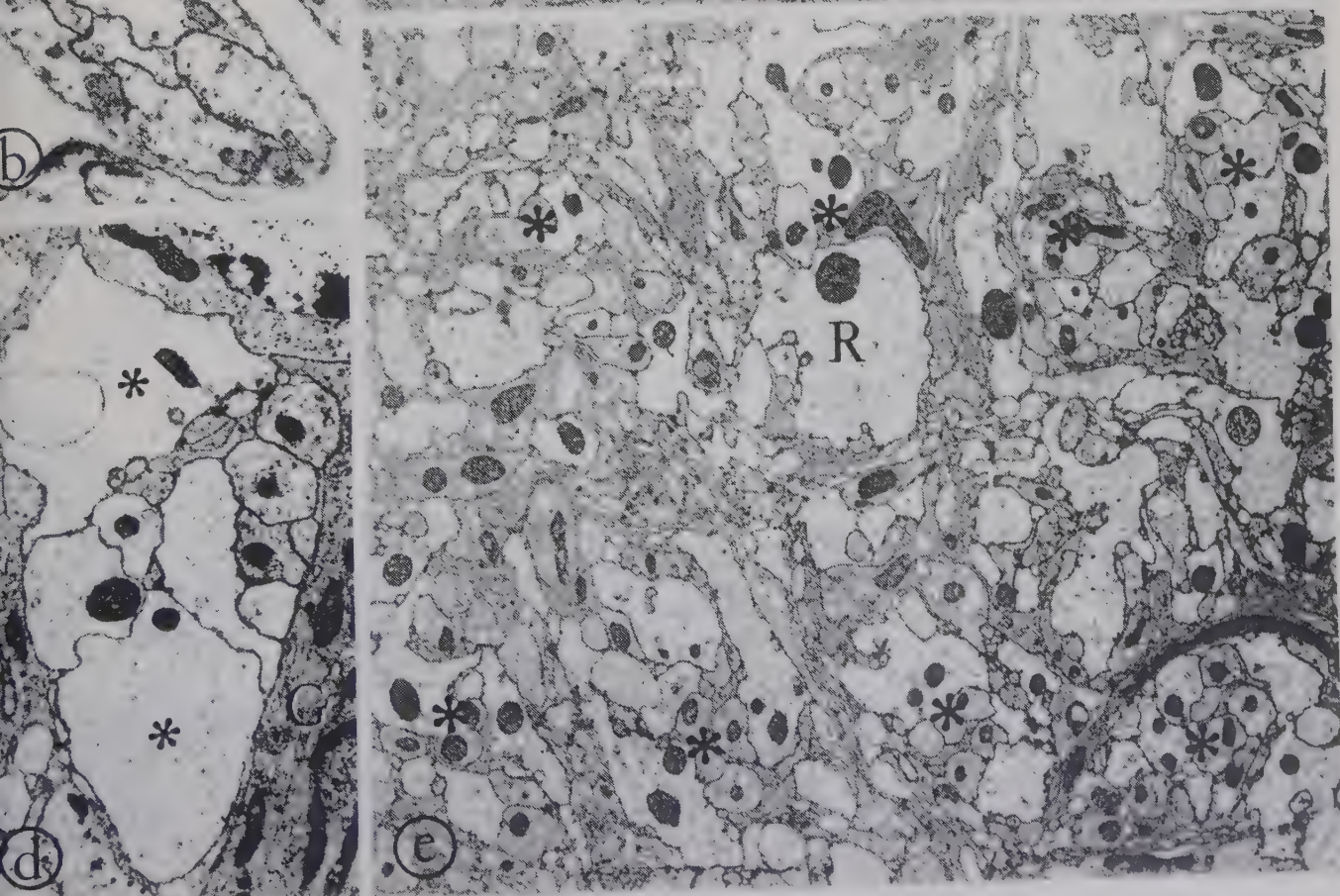
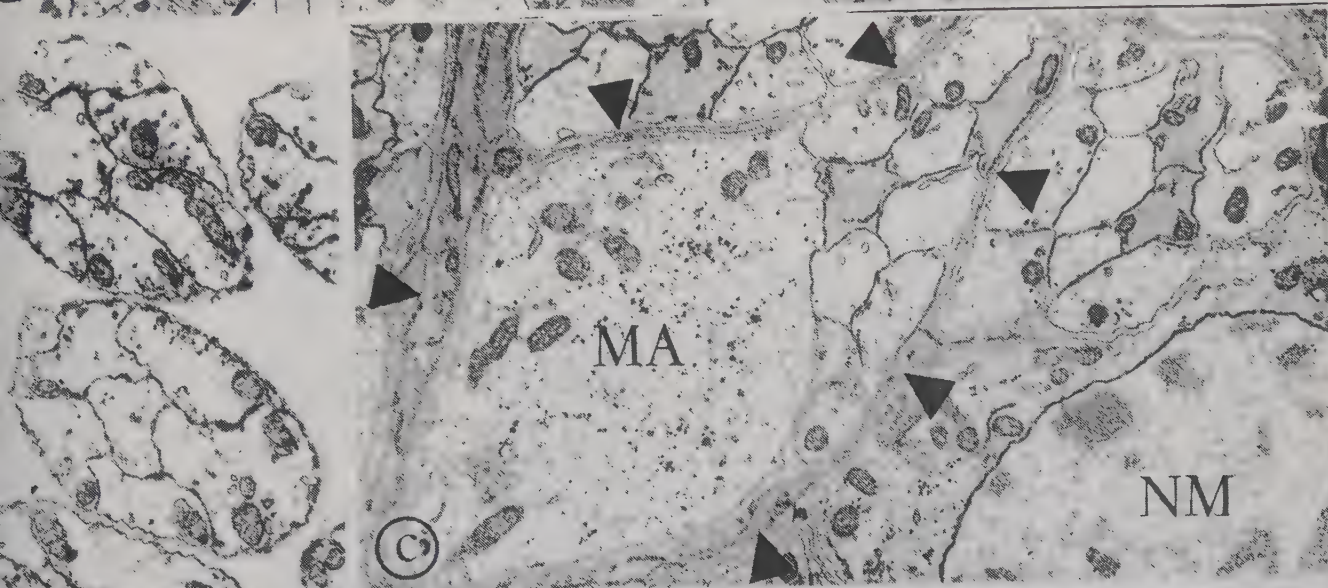
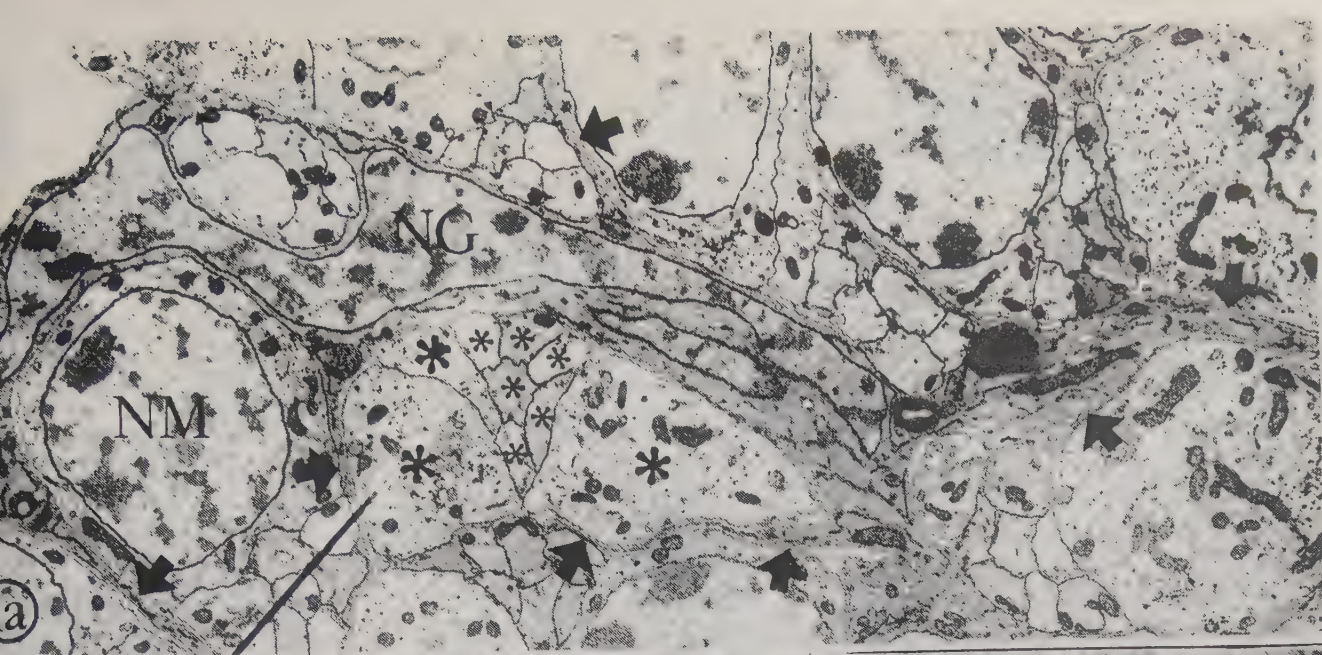
Fig. 3d A long synaptic bar with aggregation of clear vesicles. Figs 3c and 3d might represent the same synapse type in two section directions.

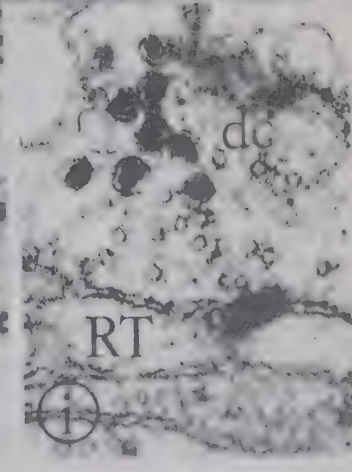
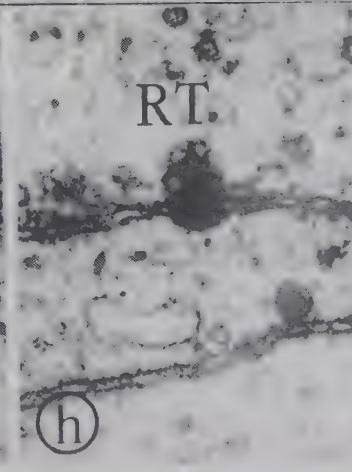
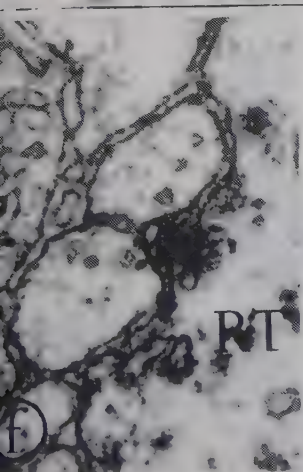
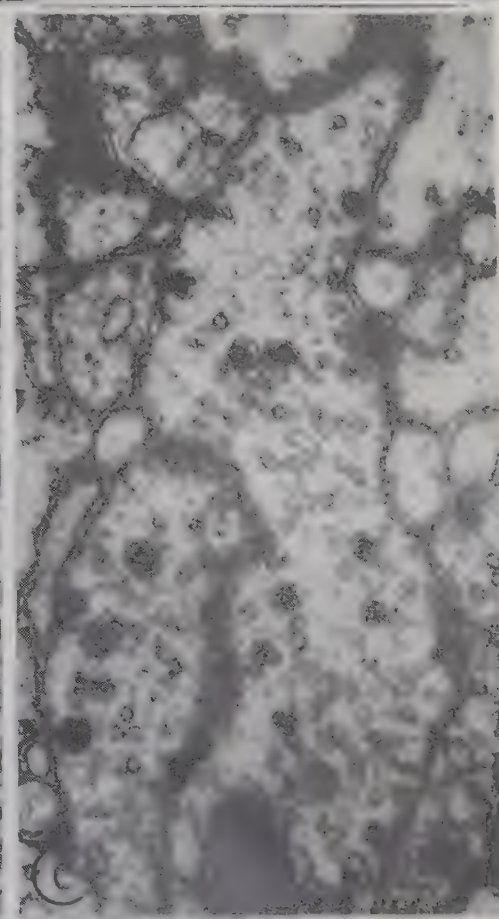
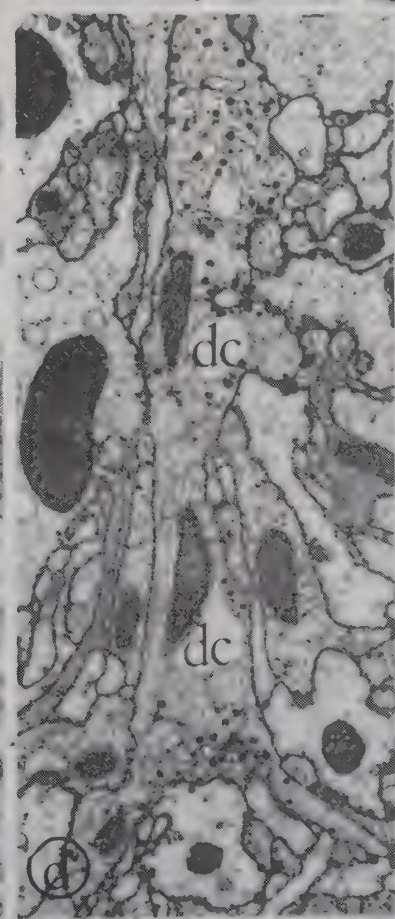
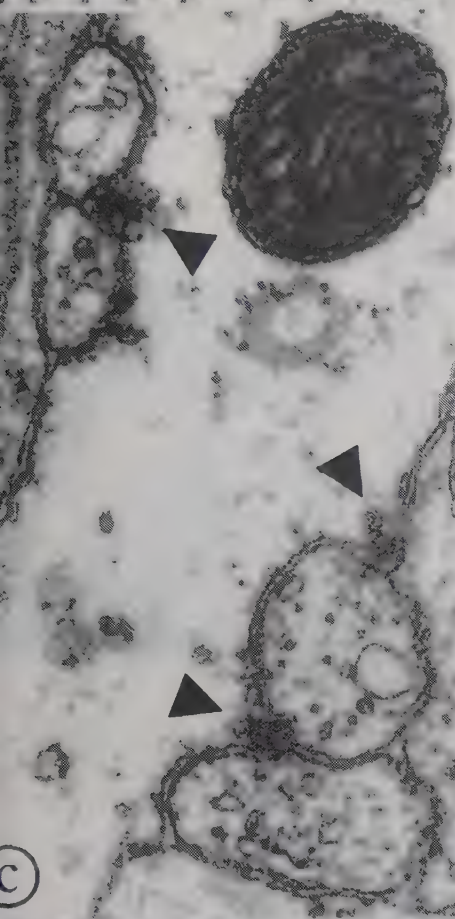
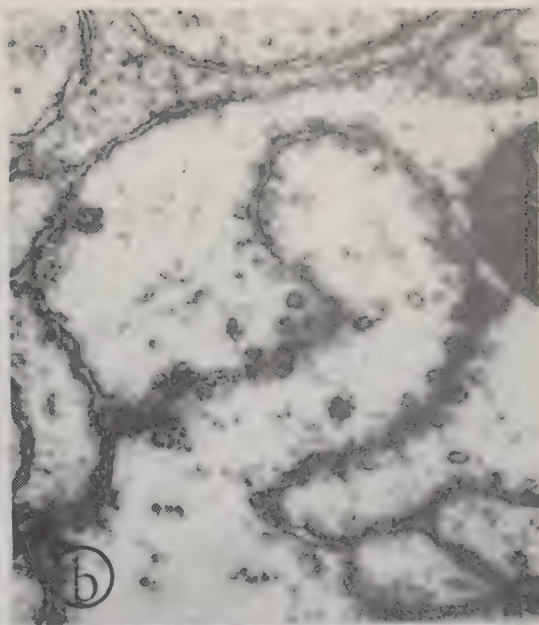
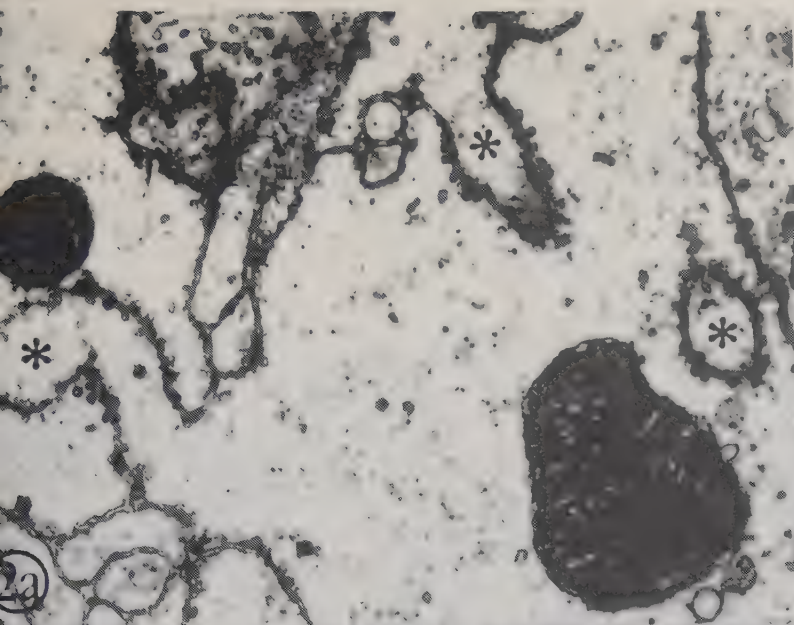
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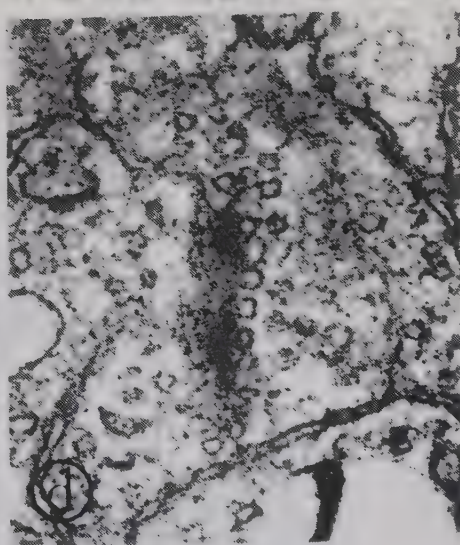
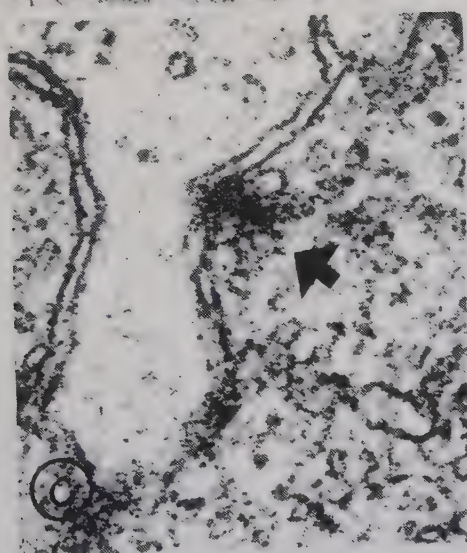
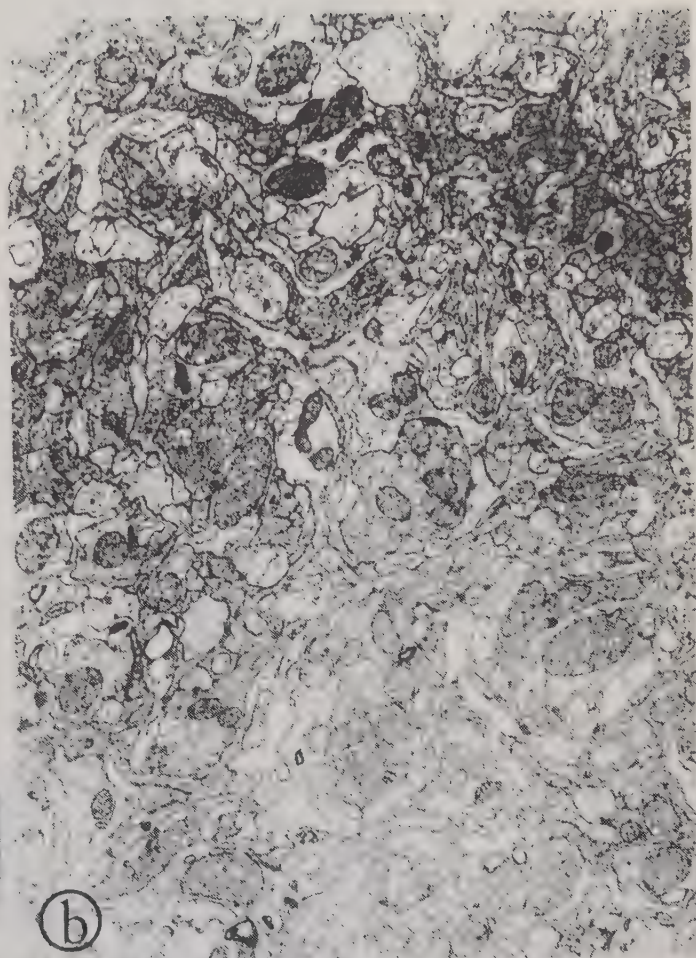
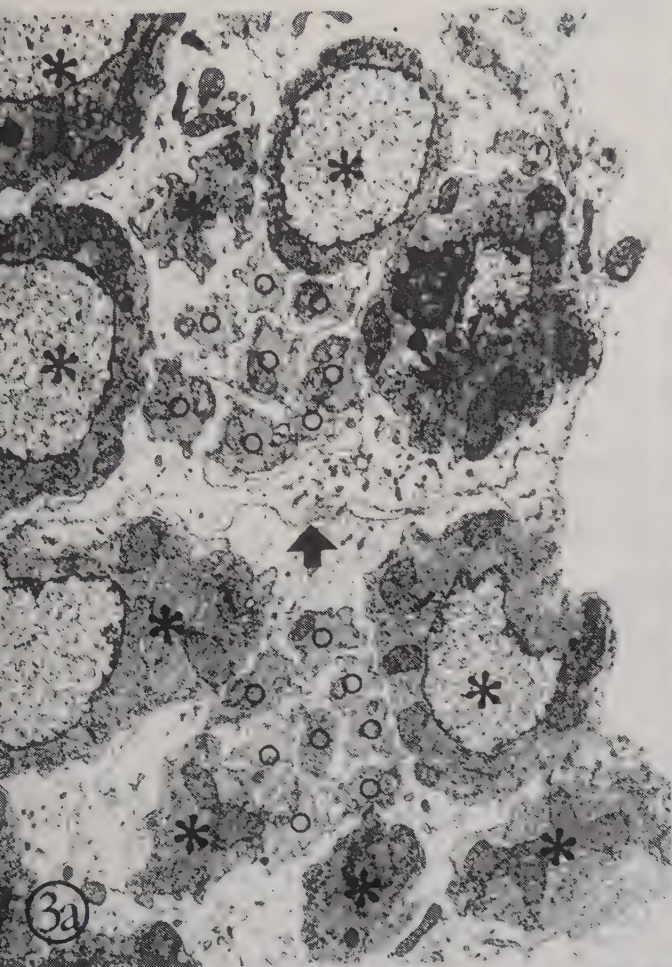
Fig. 4 Diagram of Artemia lamina monopolar neurons and receptor axons. lcl = lamina cellbody layer, epl₁ and epl₂ = strata of lamina plexiform layer. RT₁ and RT₂ = photoreceptor terminals. RL = long photoreceptor axon.

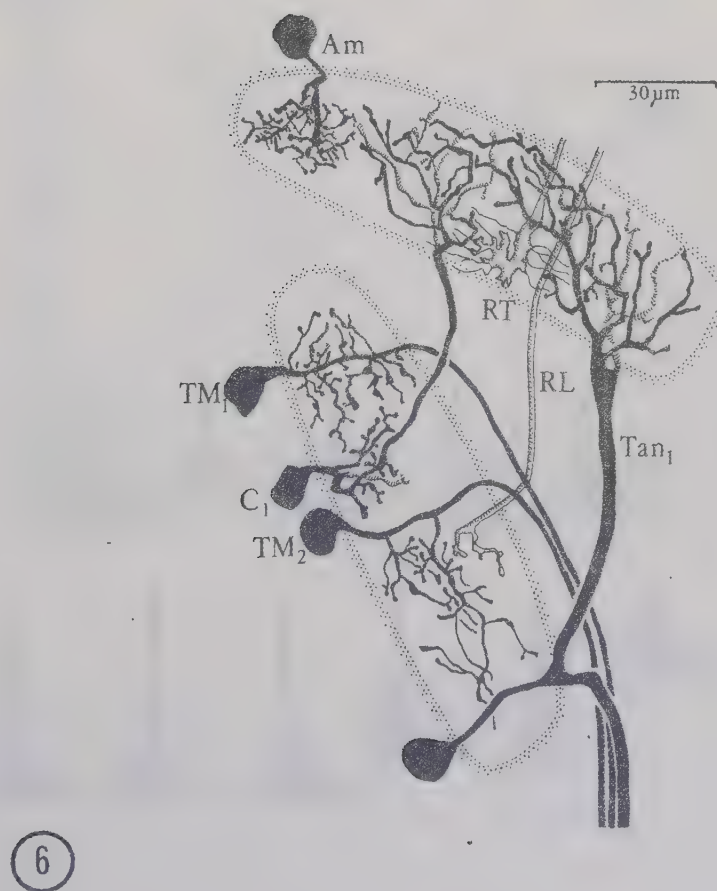
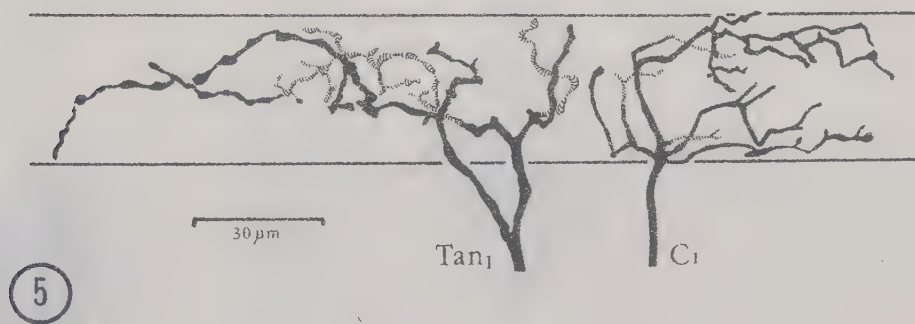
Fig. 5. Artemia lamina tangential (Tan₁) and centrifugal (C₁) neurons. Only the lamina portion of the neurons is shown.

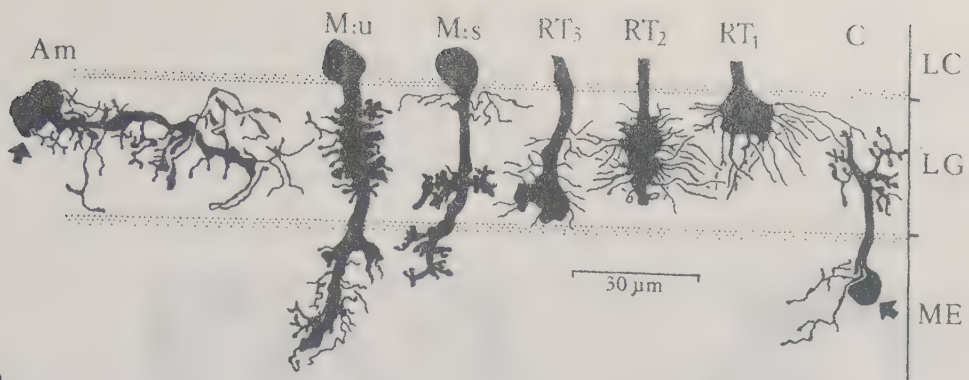
- Fig. 6 Summary diagram of some lamina and medulla neurons in Artemia. Am = amacrine neuron. RT and RL = photoreceptor axons. C_1 = centrifugal neuron. Tan_1 = tangential T-neuron connecting the lamina and protocerebrum. TM_1 and TM_2 = transmedullary neurons.
- Fig. 7 Diagram of Daphnia lamina monopolar, centrifugal, and amacrine neurons and photoreceptor axons. Am = amacrine neuron (arrow indicates cellbody located lateral to the lamina neuropil). M:u = unstratified monopolar neuron. M:s = bistratified monopolar neuron. RT_1 - RT_3 = receptor axon terminals. C = centrifugal neurons (arrow indicates cellbody). LC = lamina cellbody layer. LG = lamina plexiform layer. ME = medulla plexiform layer.
- Fig. 8 Four Daphnia receptor axons stained in the same section. All processes from the terminals are not drawn. The stratification (1-3) can be seen.
- Fig. 9 Tangential T-neuron in Daphnia connecting the lamina and the medulla with protocerebrum. Arrow indicates the cellbody.
- Fig. 10 Diagram of Artemia and Daphnia receptor terminals showing variation of arborization patterns. Upper row Artemia receptor terminals of RT_1 -type. Lower row Daphnia receptor terminals. The three terminals to the left in the lower row are variations of RT_3 axons. Note the distribution of the thin lateral processes. The next two terminals are of RT_2 type and the one to the extreme right of RT_1 type.
- Fig. 11 Semischematic drawing from electron micrograph showing the distal part of the lamina in Artemia. The upper portion of the figure shows pseudocartridges. In the lower part the periodic organization is difficult to disclose. The stippled area shows neuroglial processes. The dotted profiles are fibres with small dense cored vesicles. MN = nuclei of monopolar neurons. Asterisks show receptor terminals in pseudocartridge also shown in Fig. 1d.



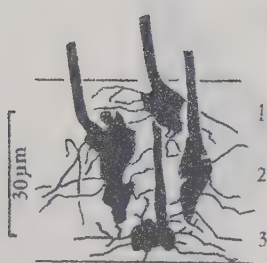




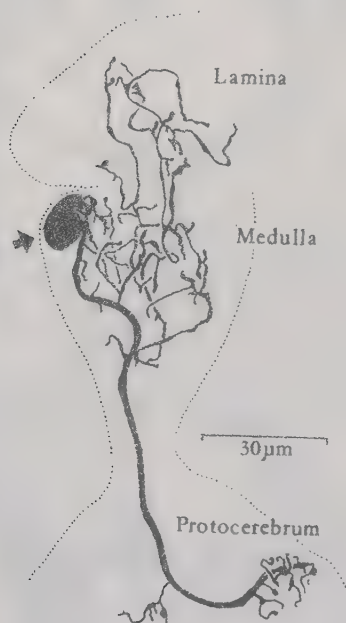




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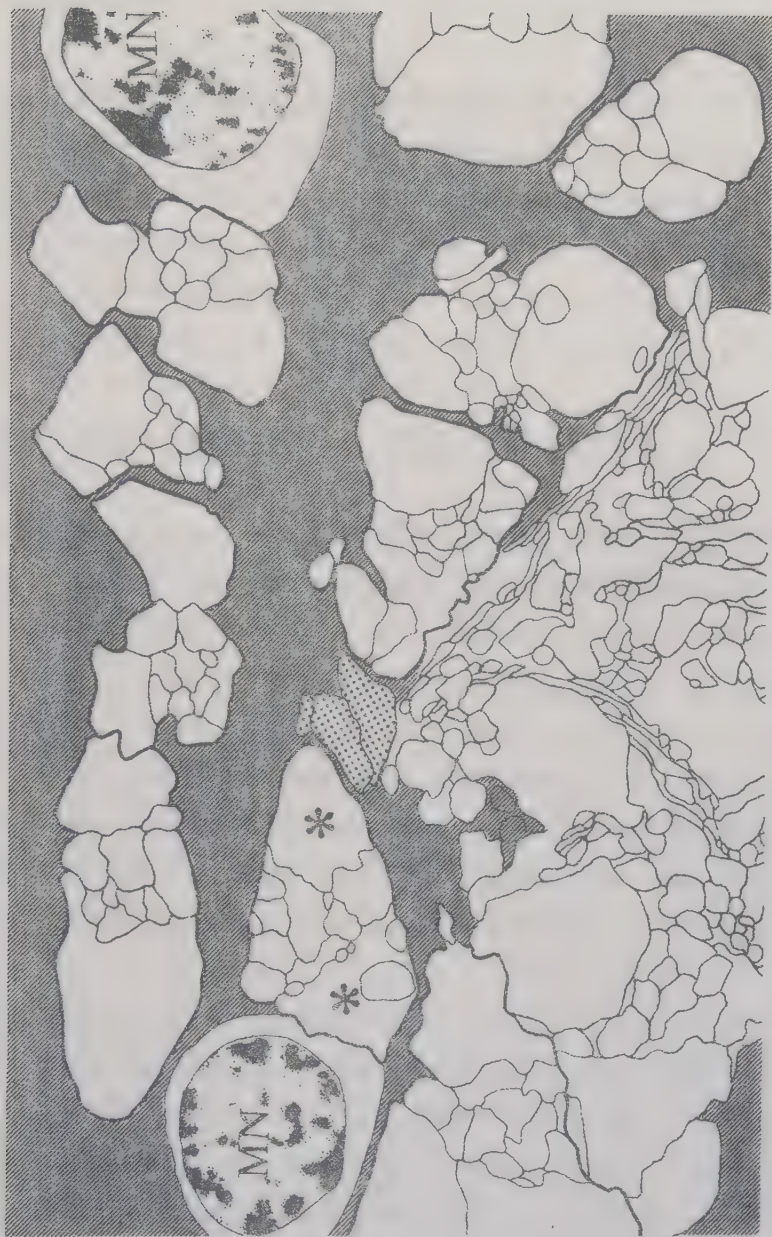
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The retina is divided into two mirror symmetrical halves with a symmetry axis along the horizontal equator of the retina. This mirror symmetry will not affect the microvillar directions of the reticular cells and has most likely no functional importance (see also Kunze 1968).

Near the basement membrane all eight receptor cells are transformed into axons. The R8 axon can still be distinguished from R1-R7 axons since the latter contain screening pigment granulae and have larger diameters. The axons of R1-R7 terminate in the first optic neuropil, the lamina ganglionaris, while R8 terminates in the second neuropil, the medulla externa.

Functional properties of the crayfish retina

Spectral sensitivity. Wald and Hubbard (1957) demonstrated with digitonin extracts that the visual pigment in the lobster was a rhodopsin with a $\lambda_{\max}$ of 515 nm. Later Wald (1967) demonstrated two light sensitive pigments in the crayfish (Procambarus and Orconectes) one with a $\lambda_{\max}$ at 515 nm, the other at 510 nm. The former was suggested to be the yellow-green sensitive rhodopsin (showed in ERG, see below), while the function of the latter still is obscure. No pigment was found corresponding to the violet sensitivity peak in the ERG.

Kennedy and Bruno (1961) used ERG to study the spectral sensitivity of the crayfish photoreceptors and found a maximum sensitivity at 565-570 nm. But with red light adaptation a second peak appeared at 425-445 nm (Goldsmith and Fernandez 1968, Wald 1968). These studies with selective adaptation of the ERG show that two colour receptors are present in the crayfish.

Later Nosaki (1969) and Waterman and Fernandez (1970) made intracellular recordings from the photoreceptors and confirmed the above findings of two classes of colour sensitive receptors. It was also concluded that the majority of the cells were yellow-green sensitive and only a smaller fraction violet sensitive.

Using extracellular recording techniques it can be further demonstrated that higher neurons in the optic nerve receive inputs from violet and yellow-green receptors (Trevino and Larimer 1970, Woodcock and Goldsmith 1970).

With microspectrophotometry (msp) of single rhabdoms in vitro a broad absorption band (525-530 nm) is shown. No pigments are demonstrated in the main rhabdom with absorption in the violet region of the spectrum (Fujimoto et al. 1966, cited in Goldsmith 1977a, Waterman et al. 1969, Goldsmith 1977a, Kong and Goldsmith 1977).

The discrepancy between the msp and electrophysiological values of the yellow-green pigment (525-530 nm and 565-570 nm respectively) can be attributed to the filtering effect of the screening pigment (Goldsmith 1977b, Kong and Goldsmith 1977). This effect can also explain the great spread of the $\lambda_{\max}$ (538-634 nm) achieved by means of intracellular recordings (Nosaki 1969, Waterman and Fernandez 1970).

Sensitivity in the near ultra violet (c. 380 nm) has been reported in one decapod crustacean only, Palaemonetes, (Goldsmith and Fernandez 1968) in contrast to the widespread occurrence in insects.

The location of the violet and yellow-green receptors in the ommatidia is by no means clear. There are apparently fewer violet sensitive cells in the ommatidia than there are yellow-green as indicated by low percentage of impalements with micro electrodes of the former: c. 10 % (Nosaki 1969) and 19 % (Waterman and Fernandez 1970).

Since Goldsmith (1977a) demonstrated that no pigment with $\lambda_{\max}$ in the shorter wavelengths is present in the main (R1-R7) rhabdom, these findings might indicate that R8 is the violet receptor and R1-R7 are the yellow-green receptors. At present no pigment is, however, measured in the R8 rhabdomere.

Polarization sensitivity. The dichroic properties of rhodopsin were discovered by Schmidt (1934, 1935, cited in Moody 1964). Since insects are known to be sensitive to the e-vector of lineary polarized light (see review by von Frisch 1965), Stockhammer (1956) studied the birefringence and dichroism of rhabdoms of hymenopterous and dipterous insects. Dichroism of the rhabdoms was considered the most likely mechanism for polarized light analysis (Moody 1964) and was suggestive because of the rhabdom's birefringence and orderly arranged microvilli of the rhabdom (Wolken et al. 1956, Goldsmith and Philpott 1956). The receptor cells would be maximally sensitive to polarized light with the e-vector parallel to its rhabdomere microvilli.

When finding sets of orthogonally arranged microvilli in the lobster, Rutherford and Horridge (1965) suggested a similar mechanism with two channels for e-vector analysis.

Using microspectrophotometry, Waterman et al. (1969) found a dichroic ratio of 2 in isolated crayfish rhabdoms. The two classes of cells with orthogonal microvilli would thus have differential sensitivities to the e-vector of polarized light. The dichroic ratio 2 infers that the chromophore dipoles lie parallel to the membrane surface but otherwise randomly oriented as suggested for cephalopods (Moody and Parriss 1961).

Electrophysiological measurements have, however, revealed much higher polarization sensitivity ratios of up to 9-12 (see below). Such high ratios indicate that the dipoles are orderly aligned in the membranes (see Goldsmith 1975).

It can be remarked that polarization sensitivity is considered to be a by-product of an adaptation to maximise absolute sensitivity of the receptors in a rhabdom (Laughlin et al. 1975. The same authors also show that in the fused layered rhabdom (so called decapod type of rhabdom),

the dipoles should be highly aligned within the plane of the membrane to have the highest absorption of unpolarized light.

Shaw (1966) demonstrated with intracellular recordings two groups of receptor cells in Carcinus with maximum polarization sensitivity (PS) 90° apart. The same results were later obtained also in the crayfish Astacus (Shaw 1969). The PS ratio was as high as 9.

With selective adaptation of the ERG with polarized light, Waterman and Horch (1966) also found two classes of polarization sensitive cells in the crab Cardisoma and mentioned similar results on a crayfish.

Waterman and Fernandez (1970) and Muller (1973) demonstrated that the PS overlaps the λ sensitivity in the crayfish so that both violet and yellow-green receptors have high PS. There would thus be no special $\lambda_{\max}$ for PS as is shown in several insects (where PS is normally in the near UV) (Wehner et al. 1975).

Recordings from higher order neurons in the medulla of a crab (Legget 1976), and the optic nerve of a crayfish and a stomatopod (Yamaguchi 1967, Yamaguchi et al. 1976) demonstrate that some neurons are sensitive to a rotating e-vector. These units have inputs from both classes of e-vector sensitive photoreceptors and do not respond to a stationary polarized light stimulus.

The PS and dichroic properties of R8 are not studied yet. With a dichroism as that of R1-R7 cells, R8 would be maximally sensitive to horizontally polarized light. Considering R8 as the only violet sensitive cell type, the violet system would be sensitive to horizontally polarized light only. Then PS would not overlap λ -sensitivity completely and there would be a third PS analyzing channel with its output directly in medulla externa.

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In this context it might be mentioned that all studied crabs possess R8 cells with orthogonally arranged microvilli, thus cancelling out PS in these cells (Eguchi and Waterman 1973, Nässel and Waterman unpublished).

Finally the R8 rhabdom of the crayfish might act as a lateral polarization filter enhancing the sensitivity of the cells with vertical microvilli in the R1-R7 rhabdom as suggested in the tiered rhabdoms of some insects (Snyder 1973a, b).

Range of absolute sensitivity. The range of sensitivity is at least 4 log units (and the dynamic range is 1.7 log units) in the crayfish retinular cells (Glanz 1968, 1971) and adaptational mechanisms are present altering the sensitivities.

Three types of adaptational mechanisms are possible (Walcott 1975):

1. Attenuation before the light reaches the receptors.
2. Adaptations within the receptors: bleaching of the photopigment and membrane changes associated with the transduction process.
3. Neural adaptation.

In addition to these adaptational mechanisms, the sensitivity range can depend on presence of photopic and scotopic receptors.

The first type of adaptational mechanism is dependent on so called photomechanical changes. Bernhards (1916) and Parker (1932) studied pigment migrations in the retinular and pigment cells of the crayfish. The distal screening pigment migration is hormonally controlled (Welsh 1941 cited in Kleinholz 1961) whereas the mechanism of retinular cell screening pigment migration is unclear. Ludolf et al. (1973) suggest a neural control of this pigment migration, i.e. direct control over the pigment by the receptor as a function of light stimulus.

The positions of these pigments in light- (LA) and dark adaptation (DA) respectively allow different acceptance angles for the individual receptors of the crayfish. In LA the angle is $4^{\circ} \pm 2^{\circ}$ and in DA $24^{\circ} \pm 8^{\circ}$ (Walcott 1974). Light entering several facets can reach one individual receptor in the LA state (Shaw 1969b) and this is accentuated in DA (Walcott 1974). Walcott (1974), however, found no correlation between the relative sensitivity of a unit to a point source of light and the acceptance angle.

Other photomechanical changes have not been suggested in the crayfish (cp. insects (Walcott 1975)).

It can be mentioned, however, that the crabs Ocypode and Grapsus show extreme changes in size of their rhabdoms in LA and DA. The diameter of the rhabdom in DA is more than three times larger than that in LA. This size difference is due to increasing number, length, and diameters of microvilli. The changes occur within 12 hours with minimum rhabdom diameter at noon and maximum at midnight in a 12:12 light-darkness cycle (Nässel and Waterman in prep.).

Adaptations within the receptors themselves are shown for the crayfish by Glanz (1968, 1971). He studied both LA and DA of the photoreceptors using intra- and extracellular recording techniques. DA was followed by a gradual repolarization sensitizing the receptors, while LA desensitized the receptors.

When the eye was permitted to dark adapt after exposure to an intense flash, the response to a second flash increases as a function of time in darkness. Glanz (1971) also studied adaptation in central visual neurons and found that their adaptation was only a function of the receptor adaptation.

Since no recordings are made from R8, their absolute sensitivity cannot be compared to that of R1-R7. It is, however,

possible that R8 has a lower sensitivity than R1-R7. In the dipterous insects the R7/R8 cells have such a lower absolute sensitivity together with a different λ_{max} and a smaller acceptance angle (Kirschfeld 1973, Harris et al. 1976, Heisenberg and Buchner 1977). Thus, two functional subsystems are present in the fly retina (Kirschfeld 1973).

In the crayfish the R1-R7 system could be a high sensitive (with a broader sensitivity range) and R8 a low sensitive (narrower sensitivity range) system. The functional relations between the two systems and their mediation of for example optomotor responses remain to be studied.

2. RETINA-LAMINA PROJECTION (Nässel 1976a)

Early it was discovered that the ommatidial mosaic in the arthropod retina was impressed on the first optic neuropil (Viallannes 1891). The lamina columnar units were termed neurommatidia (Viallannes 1891) or cartridges (Cajal and Sanchez 1915). Vigier (1908) and Cajal and Sanchez (1915) showed with Golgi preparations that the receptor axons from the ommatidia project on the lamina in specific patterns in insects. The most spectacular pattern was found in the fly (Vigier 1908) where axons from one ommatidium project on six laminar cartridges and reversely one cartridge receives axons from six ommatidia. These findings were later confirmed in more detail (Trujillo-Cenoz and Melamed 1966, Braitenberg 1966, Horridge and Meinerzhagen 1970). Kirschfeld (1967) found that axons from six receptor cells with rhabdomeres with the same optic axis in six different ommatidia converged to one cartridge, thus forming a basis for neural superposition.

Present data show that insects with split rhabdoms (Dipterous and Hemipterous insects) have divergent axonal projection, i.e. axons go to more than one cartridge from each ommatidium.

On the other hand, insects with fused rhabdoms have a direct retinotopical projection, i.e. the axons from one ommatidium go directly to the underlying cartridge (Meinertzhagen 1976).

With this background it is intriguing to study the projectional pattern of receptor axons in crustacean compound eyes. Previously no detailed studies have been attempted on retinalamina projections except in the cladoceran Daphnia (Macagno et al. 1973). Hanström (1928) showed in his schematic drawings from Golgi preparations that receptor cell axons crossed each other distal to the lamina in decapod crustaceans, but gave no detailed pattern.

The distance between the retina and the lamina is very long in decapods (up to one mm) and the axons are often closely packed together and take bent courses. Obviously this has hindered a serial reconstruction and complete tracing of receptor axons.

Already Viallannes (1891) and Parker (1897) demonstrated a challenging axonal rearrangement at the basement membrane (BM) below the retina in the crayfish and the lobster. The pattern which was later confirmed in other decapods (Kunze 1967, Meyer-Rochow 1975) was such that axons from one ommatidium split and penetrate the BM through four different meshes. These meshes were also penetrated by axons from neighbouring ommatidia.

This rearrangement might be suggestive for a divergent axonal projection pattern in decapods in spite of fused rhabdoms.

In order to unravel if this was the case, EM fixed compound eyes of the crayfish Pacifastacus were serially cross sectioned and compared with silverpreparations. The receptor axons were traced through the basement membrane to a level where the axons are closely packed together and due to their heavy pigmentation are difficult to trace (cp. crabs below).

The following pattern was found. One ommatidium sends its axons to four meshes of the BM where they join axons from four neighbouring ommatidia. The four meshes contain the following axons: R1, R4, R5: R2, R3, R6, R7, R8: R4, R5, R1: R6, R7, R8, R3, R2. The underlined axons stem from one parent ommatidium.

The groups containing three and five axons respectively will fuse below forming bundles of eight. Each such bundle comprises one representative each of the axons R1-R8, but from three ommatidia. Conversely each ommatidium sends axons to three such bundles. The axonal distribution is such that the eight axons represent receptors with both microvilli directions in each of the three participating ommatidia.

As previously mentioned there is then a region of some hundred μm where the axons are densely packed and no attempts have been made to trace the axons. In this region it is highly possible that new constellations of axons are formed. The arrangement achieved below the BM can even be reversed resulting in groups of eight, each originating from one ommatidium only.

After the region with densely packed axons, the axons become thinner above the lamina cellbody layer, and groups of eight axons reappear. Whether the ommatidial groups of eight axons rejoin or the subbasement membrane groups of eight persist remains to be demonstrated.

These bundles of eight reticular cell axons are in the cellbody layers distal to the lamina joined by the axons of monopolar neurons. So called pseudocartridges are formed. The axons of the pseudocartridges enter the lamina where they form synaptic compartments, cartridges. No crossing of axons normally occurs in this region distal to the lamina as indicated by Hanström (1928).

If the axons in the bundles of eight formed below the basement membrane indeed are the ones found in the cartridges, then one cartridge receives axons from three ommatidia and one ommatidium sends axons to three cartridges.

In the light of recent investigations of other crustaceans and insects a projectional pattern of retinotopic type (one ommatidium projects to one cartridge) would be expected in the crayfish. This is the case in Daphnia (Macagno et al. 1973) and probably is the case also in Artemia (Nässel et al. 1977). In mysids the axons from one ommatidium penetrate the BM in three meshes without joining axons from adjacent ommatidia and the axons form bundles of seven or eight from one ommatidium only (Hallberg 1977).

In Leptograpsus Stowe (1977b) demonstrated that one ommatidium sends its axons to one underlying cartridge. This could be demonstrated with serial sectioning since the axons from about thirty ommatidia are collected in large bundles (and are less pigmented) and not as in Pacifastacus are densely packed together.

Neural superposition in the crayfish compound eye seems less likely since its fused rhabdoms have divergent optical axes, and optical superposition is demonstrated (see previous chapter). Sampling of angular information at the retinal level would not be directly conserved at the laminar level with a divergent axonal projection. There is thus no direct functional explanation for a divergent axonal projection as hypothesized by Nässel (1976a), but rather a retinotopical projection seems more probable even in the crayfish.

It is, of course, remarkable that the axons penetrate the BM in a complicated pattern just to be reversed deeper down. This could, however, be an effect of developmental mechanisms so that for instance the formation of the basement membrane results in a divergence of axons which has to be reversed further down. This was also suggested by Stowe (1977b), who

also recognized that the extensions from the crystalline cone cells separate the axons in four groups. There is actually indications that these extensions form the cellular part of the BM in Artemia (Elofsson and Odselius 1975). In Pacifastacus the four cone extensions form flat feet below the rhabdom and together with extensions from the two distal pigment cells they form the cellular part of the BM (Nässel unpublished). No special cells are found forming the BM as described in Astacus by Krebs (1972). It is therefore as Stowe (1977b) suggests likely that the crystalline cone extensions separate the axons when forming the BM and that this grouping has no functional significance.

3. TYPES AND ARRANGEMENTS OF NEURONS IN THE LAMINA (Nässel 1976a, 1977)

General organization of the lamina

The lamina is the outermost optic neuropil receiving the photoreceptor axons. Here the photoreceptor-first order neuron synapses are found.

The lamina is composed of two cellbody layers distally, a plexiform layer (epl), a proximal cellbody layer and glial layers enwrapping the neuropil. The lamina is connected with the medulla externa (ME) with axons forming a horizontal chiasma. The lamina neuropil is vascularized by numerous capillaries from sinuses distal and proximal to the neuropil. The lamina neuropil has a columnar organization with modules termed cartridges. The plexiform layer is divided in two sublayers, epl₁ and epl₂.

Types of neurons

The neurons in the lamina can be divided into five classes: photoreceptor axons, monopolar neurons, tangential neurons, centrifugal small field neurons, and multipolar cells.

Photoreceptor axons. The photoreceptor axons are of three types: one type is characterized by enlarged terminals either distally in epl_1 , or proximally, in epl_2 ; the second has a terminal in epl_1 but a slender elongation ending in epl_2 ; and the third type of axon terminates in the medulla externa.

Monopolar neurons. The monopolar neurons have their cellbodies distal to the lamina plexiform layer, and axons branching in different patterns in this layer. Their axons terminate in medulla externa. Five types of monopolars are distinguished (M1-M5). Two types are stratified (M3 and M4) with lateral processes corresponding to the receptor terminal strata, M3 in epl_1 and M4 in epl_2 . The remaining monopolar neurons are unstratified (i.e. have lateral processes in both lamina layers), two with a small field (M1 and M2) and one with a large field over several cartridges (M5).

The monopolar neurons have their cellbodies in either of the two cellbody layers, distal to the plexiform layer, M1 in the inner cellbody layer and M2-M5 in the outer.

The branching patterns of the monopolar neurons vary within the cell types depending on the location of their axons in the lamina and their location in the cartridge (see below).

Tangential neurons. Tangential neurons of two main types (Tan 1 and Tan 2) are found with the Golgi technique. A third type revealed with the Falck-Hillarp fluorescence technique will be described in the next chapter. Tangential neurons are characterized by long lateral lamina processes (extending over several cartridges) and an axon with a terminal in ME. Their cellbodies are situated in the chiasma between the lamina and ME and are connected to the axon by means of cellbody fibres, thus forming T-neurons (as defined by Strausfeld and Blest 1970).

Tan 1 has a large number of long lateral lamina processes. These processes are branching extensively in both epl_1 and epl_2 and about 20-30 cartridges will be within reach. The terminal in the medulla is situated shallowly and has a small field arborization.

Tan 2 has a much larger lateral extension in the lamina (around 100 cartridges). The main lateral branches are situated below the epl_2 . From these main branches secondary branches project laterally and from these and the main branches tertiary processes reach up into the lamina plexiform layer. These tertiary processes (approximately one per cartridge) in their turn have lateral processes in the plexiform layer. Two types of tertiary processes have been revealed, one with a basket like arborization, the other with a wide field diffuse arborization. It is not clear whether there thus are two types of Tan 2 neurons: one with basket like, the other with diffuse tertiary processes, or if this is just another variation within a neuron type. The Tan 2 medulla terminal is extensively branching over a large field in a shallow part of the medulla neuropil.

For both Tan 1 and Tan 2 it has proved impossible to stain the cellbodies with the Golgi technique. Only the cellbody fibres are resolved.

Centrifugal small field neurons. A type of neuron is found that has a small field arborization in the lamina plexiform layer. It has no cellbody distal to the lamina and can easily be distinguished from a monopolar neuron by its arborization pattern. It is referred to as a medulla-lamina centrifugal neuron by analogy with similar neurons found in insects (Strausfeld 1976) and in crabs (Stowe et al. 1977). Its axon can be followed from ME, but its ME anatomy and cellbody location is not revealed.

Multipolar cells. Two types of multipolar cells are located in the lamina, MP 1 and MP 2. MP 1:s have their cellbodies

distal to epl_1 and MP 2 proximal to epl_2 (in the proximal cellbody layer, PCL). From the cellbodies numerous processes invade the plexiform layer. The lamina processes have lateral spines or lamellar specializations and sometimes basket like arrangements are formed. The basket arrangement corresponds in size to one cartridge. These multipolar cells can be either of neural or neuroglial nature. With the LM technique applied here it has been impossible to solve this problem.

The laminar specializations of the MP processes could well correspond to the thin glial profiles found in conventional EM. The extensive overlap of MP:s with several processes from 3-6 MP:s to each cartridge could result in a glial sheath resembling the one found in EM.

On the other hand, large field amacrine cells are found in several insects (Strausfeld 1976), and branchiopod crustaceans (Nässel et al. 1977) and it is possible that also decapod crustaceans possess such cells. The anaxonal MP cells are then likely candidates for amacrine cells.

Arrangements of neurons

Using Golgi and Bodian preparations for light microscopy, connectivity patterns cannot be clarified, but when resolving branching patterns and stratifications, some ideas of neural relations can be achieved.

The columnar organization with cartridges and bistratification of the lamina plexiform layer has already been mentioned as organizational patterns. The cartridges, which will be discussed at length in chapter 5, are synaptic units formed by seven photoreceptor terminals in two layers and 3 or more monopolar neurons. Since each cartridge will represent a smaller part of the visual surround (ultimately one ommatidium) it is interesting to clarify which neurons that derive their input from one cartridge only.

With light microscopy it is clear that three of the monopolar neurons (M2-M4) each has one representative in each cartridge. M1 axons are not situated in the cartridges proper. M5 cells are probably present in a fraction of the cartridges only. With its lateral width of c. 100 μ m they can reach 5-10 cartridges. These cells might then be present in a ratio of 1:10-1:5 to the cartridge number.

The tangential neurons interact with the elements of a larger number of cartridges. The signal directions in the tangential neurons and the elements they interact with cannot be clarified with LM. These neuron types can be either centripetal or centrifugal, and can probably also form local circuits in the lamina. There is an extensive overlap between Tan 1 neurons and probably also Tan 2:s overlap.

In chapter 5 the connectivity patterns of the lamina neurons will be discussed in more detail.

4. AMINERGIC NEURONS

(Elofsson, Nässel, Myhrberg 1977)

Elofsson and Klemm (1972) demonstrated a specific pattern of green fluorescence in the crayfish lamina and medullae with the fluorescence histochemical method of Falck-Hillarp (Falck 1962, Björklund et al. 1972). It was also concluded that this fluorescence represented a catecholamine (Elofsson and Klemm 1972).

With this method normally only the **terminal** portions and the cellbodies fluoresce and no information on the morphology of the whole neurons is achieved. This is due to the fact that the reaction product is bound to the content of small dense cored vesicles presumed to be storage sites for the catecholamines (Hökfelt 1968, de Robertis 1969), and these are accumulated terminally and in the cellbody.

With the introduction of exogenous catecholamines after reserpine treatment the neural uptake is cytoplasmic, however, and the histochemical fluorescence method reveals the whole neuron.

Thus, with repeated reserpine treatments the catecholaminergic neurons were depleted and subsequently exogenous amines were introduced by incubation of excised neuropils. The two amines α -methylnoradrenaline and dopamine respectively were used in the experiments.

With this incubation intense fluorescence appears in the lamina plexiform layer, the external chiasma, and in the same layers as normally in the medulla. It was thus possible to reveal the entire fluorescent neurons in more detail and the reconstruction of a new neuron type was possible. This neuron type was designated: catecholaminergic tangential neuron, CTN (see below).

This fluorescent neuron is of tangential T-neuron type. The lamina portion consists of an axis fibre with lateral processes at the distal and proximal margins of the plexiform layer. These processes probably extend over several cartridges and form part of two tangential strata in the plexiform layer. Smaller spines emerge from the axis fibre between the two tangential strata giving the neuron a blurred appearance in the fluorescence microscope.

Axons connect the lamina via the outer chiasma with terminals in the distal part of ME. Each terminal is lampbrush shaped and together they constitute the distal of the three fluorescent layers in ME.

The cellbodies of these tangential neurons lie in the cellbody region wedged in the outer chiasma. The cellbody fibres are not resolved in the present technique, but probably they connect the cellbodies with the axons in a T-neuron fashion.

There is no neuron type yet classified in the Golgi technique that can be correlated with the fluorescent neuron. It is not rare that certain cell types are more resistant to the Golgi technique (see Boycott et al. 1975).

In EM two types of fibres containing dense vesicles are displayed in the untreated (normal) lamina. Type 1 has true dense cored vesicles with a diameter of c. 60-80 nm. Type 2 has larger vesicles (100-160 nm) entirely filled with electron opaque content. Both types of fibres also contain clear vesicles (diameter c. 20-40 nm). Type 1 and 2 fibres are generally found together in bundles.

Tangential processes of type 1 and 2 fibres are found distally and proximally in the lamina plexiform layer. Between those two strata very few such profiles are found, and below the lamina in the chiasma no profiles are seen with dense vesicles.

After reconstruction of the profiles in EM it is apparent that the type 1 profile pattern matches the fluorescence-picture

Further studies have revealed that the type 1 neurons form synaptosoid contacts with type 2 neurons in the two tangential layers (Nässel unpublished). These synaptosoid contacts are characterized by one presynaptic type 1 profile and two postsynaptic type 2 profiles. In the type 1 profile there is a small dense area around the presynaptic membrane and a cluster of clear vesicles. Also in the type 2 profiles there is dense material around the membranes. Occasionally also these profiles have clear vesicles clustered near the synaptosoid site.

Exogenous catecholamines are taken up only by catecholaminergic neurons (by a reserpine resistant mechanism) and since the fluorescence pattern resembles that of the standard technique it is quite clear that the CTN's represent catecholaminergic neurons.

Analysis of a related crayfish Astacus astacus by means of microspectrofluorimetri indicates that the green fluorescence in these neuropils is derived from dopamine or a dopamine related compound (Elofsson and Klemm 1972). On morphological grounds the type 1 fibres with dense cored vesicles are referred to as the catecholaminergic neurons and type 2 to neurosecretory fibres (Shivers 1967, Scharrer 1968).

The morphology of the presumed neurosecretory neurons is resembling the CTN's in the lamina but no further details are available as to cellbody location or connections with other neuropil regions. A smaller number of large cellbodies is located just below the lamina that might belong to a neurosecretory system (see also Hamori and Horridge 1966a).

The site of release of the presumed neurosecretion is also unclear. No close appositions to capillaries or blood sinuses have been revealed in the plexiform layer or proximal or distal to the lamina.

The synaptosoid formations between type 1 and 2 fibres make it suggestive that the release from neurosecretory neurons is controlled by catecholaminergic neurons. Since the site of release is not clear, the function of this system remains obscure. Arechiga et al. (1977) demonstrated a neurodepressing hormone in the CNS of the crayfish. Such a hormone could be released and act within the lamina neuropil.

The circadian ERG activity (Arechiga and Wiersma 1969, Page and Larimer 1972, 1975) is one mechanism that probably is under hormonal control. This ERG activity is modified by 6 OHDA injections (Barrera-Mera 1976). Thus, the ERG activity mechanism has an amacrine component. The discharge from higher order neurons (sustaining units) is blocked by 6 OHDA (Barrera-Mera 1976). There are thus reasons to believe that the type 1 and 2 neurons somehow are involved in modulating the photoreceptors or visual interneurons.

The role of catecholamines in the retina is far from clear even in vertebrates (van Harrevelde 1977). In some vertebrate retinæ, catecholaminergic neurons are found (Ehinger 1976). The function of these neurons is also obscure, though some indication is present that they depress firing of retinal ganglion cells and alter responses of horizontal and bipolar cells (for references see Ehinger and Nordenfeld 1977). Since no neurosecretory fibres are demonstrated in the vertebrate retina these aminergic neurons interact with retinal interneurons only (Dowling and Ehinger 1975, Boycott et al. 1975).

5. CARTRIDGE ARRANGEMENTS AND SYNAPTIC ORGANIZATION OF THE LAMINA

(Nässel 1976a, Nässel and Waterman 1977 and previously unpublished results)

Connections in the cartridge (Figs 1, 2)

The term cartridge was applied to the columnar units early found in various arthropod laminae. In certain insects this columnar organization is very distinct (Dipterous insects: Trujillo-Cenoz and Melamed 1963, Boschek 1970; mayflies: Wohlbürg-Buchholz 1977; a dragonfly: Armett-Kibel et al. 1977) i.e. the cartridges are separated by means of glial cells. In other insects the cartridges are less separated (bees: Ribi 1976; ants: Wehner 1976) or even difficult to resolve (cockroaches: Ribi 1977).

The crayfish cartridges are not completely separated by glial cells and are not even clearly distinguishable at all levels. Nevertheless, columnar units are present formed by seven photoreceptor terminals in two layers and four to five monopolar neurons.

With conventional EM the cross sectioned cartridge looks very irregular and disordered and the individual profiles are difficult to identify. This can easily be explained by the

irregular shapes and repeated arborizations of the lamina neurons. It is, however, possible to trace axons through the cellbody layers where they form so called pseudocartridges. These pseudocartridges which transform into cartridges in epl_1 contain eight photoreceptor axons and four to five monopolar neurons just above the epl_1 . The receptor axons are more electron dense and can easily be distinguished from monopolar axons.

Seven of these receptor axons gradually expand to large irregular terminals filled with clear vesicles. Four terminals are found in epl_1 and three in epl_2 in each cartridge.

To avoid tedious serial sectioning of the lamina neuropil for the unravelling of the lamina connectivity patterns the Golgi-EM technique was employed. This method uses previously identified Golgi-stained neurons that are reembedded and analyzed in EM. Thus, the postsynaptic relations of the stained neurons can be clarified. Since the crayfish lamina neurons have no constant shapes or locations in the cartridges (cp. the fly lamina, Strausfeld 1971) it is still a tedious reconstruction work to solve the total variety of connections. As a start, the monopolar neuron relations to the photoreceptor terminals have been studied.

Three of the monopolar neurons (M2-M4) were initially studied since they from LM appeared to be postsynaptic to the photoreceptors in each cartridge. These neurons are present in all cartridges.

The only type of synapse found where RT's are presynaptic is characterized by an evagination of the RT membrane facing three postsynaptic profiles (triads). This evagination contains a presynaptic bar and clear vesicles. Occasionally two or more triads are fused and share certain elements.

M2 has its axon more or less centrally in the cartridge and its radially arranged spines contact all presynaptic areas

of R1-R7 in $ep1_1$ and $ep1_2$. The spines are restricted to the parent cartridge only. The M2 spines always contribute as the central of the three postsynaptic profiles and they are present in all RT triads of the cartridge. Thus, the M2 neuron is postsynaptic to all seven RT's of the cartridge. Since each RT forms approximately 20 synapses the 7:1 convergence gives c. 140 synapses on each M2 from RT's (these numerical data remains to be calculated in more detail).

The M3 axon is somewhat off-centre in the cartridge and has radially arranged spines contacting presynaptic photoreceptor membranes of the four RT's in $ep1_1$ of the cartridge only. The M3 spines constitute one of the lateral elements in each $ep1_1$ triad. The result is that M3 is postsynaptic to $ep1_1$ RT's exclusively.

M4 also has its axon off-centre, but its radial processes exclusively contact the three RT's in $ep1_2$. The M4 spines participate as one of the lateral profiles in each $ep1_2$ triad in the cartridge, thus making M4 postsynaptic to $ep1_2$ RT's only. Each M3 and M4 neuron will thus receive a 4:1 and 3:1 convergent input respectively giving around 80-60 synapses. No spines have been seen invading adjacent cartridges from either of the two neuron types.

Both M3 and M4 spines form small knob-like protrusions into the RT's (diameter of the protrusions c. 0.16-0.50 μm). These invaginations are found near the synaptic triads, but also away from these sites. No specializations of the membranes or clusters of vesicles are seen in connection with these protrusions.

M1 was later studied with the Golgi-EM method (previously unpublished results). It was found that M1 neurons constitute the remaining profile in the triads in a specific pattern (Figs 1, 2).

The M1 neurons are situated peripherally in the cartridge and their spines contact a fraction of the cartridge RT's only. One of the M1 neurons studied only contacted two RT's in epl_1 and two in epl_2 and participated in all triads in those RT's.

In two cases two M1 neurons were stained in the same cartridge. Such a preparation was examined in EM. In this case all RT triads of the cartridge each contained one profile filled with silver chromate. The two M1 axons were located peripherally at opposite ends of the cartridge (cp. Pandalus: Nässel 1975).

The above findings indicate that two M1 neurons are present in each cartridge as is the case in Pandalus (Nässel 1975). One of these receives input from two epl_1 and two epl_2 RT's, and the other from two RT's in epl_1 and one RT in epl_2 . Thus, a second ~~channelling~~ of information is present in addition to the one of M3 and M4 neurons (Figs 1, 2).

So far no other inputs on M1-M4 are demonstrated than that from RT's. This does not exclude that such contacts are present. It is namely difficult to resolve synapses other than the triads with the Golgi-EM method since the membranes of the Golgi impregnated profiles are slightly distorted.

Intercartridge connections

Certain of the elements of the cartridge appear to be connected with units that have processes in several cartridges. There are several kinds of intercartridge connections possible. Units like M5, Tan 1 and Tan 2 can transfer signals centripetally from the lamina over a larger portion of the retinal mosaic. Another alternative is that Tan 1 and Tan 2 are transferring signals from the medulla centrifugally to the lamina over a large field. They can, in addition, form local circuits in the lamina and/or the medulla. Such local circuits would also be formed by MP cells if they indeed are neurons not glial cells. None of the above neural pathways have been

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clarified yet. Studies of Tan 1 and M5 with the Golgi-EM technique have shown that neither of them get direct input from RT's via the triad synapse complexes or other types of contacts (Nässel in prep.). Instead, these neurons appear to interact with other lamina elements in a complicated pattern. It seems possible that Tan 1 is actually presynaptic to lamina elements (possibly M2's) and thus is centrifugal.

Functional properties of the lamina neurons

The functional roles of the lamina connections are far from clear. Several integrative properties of the lamina neurons are studied in insects (see Laughlin 1976, Zettler and Weiler 1976), and these will first be presented.

It seems quite clear that the so called large monopolar neurons (LMC) (cp. M2 in the crayfish) that receive input from the receptors, code intensity and contrast over a wide range of intensities. Signal amplification can be achieved by means of numerous parallel synapses between the photoreceptors and the LMC's and the convergence of several (6 in the fly and dragonfly) photoreceptors on one LMC (Laughlin 1976).

Feed-back and feed-forward loops act in the receptor-interneuron chain changing the dynamic range of the LMC's in light and dark adaptation. Even the dark adapted LMC's can thus perform effectively as a high contrast coding pathway (Laughlin 1976).

The angular sensitivity of the photoreceptors is not only maintained by the LMC's, but is narrowed further by lateral inhibition (Zettler and Järvilehto 1972, Laughlin 1974a).

The synaptological substrates for these integrational properties in the lamina have been studied in the fly (Strausfeld and Campos-Ortega 1976). Pathways possibly underlying neural adaptation and lateral inhibition were described. The connections in the lamina revealed by these authors are highly complex, but they follow a very orderly and precise pattern.

Surprisingly few electrophysiological studies are performed on crustacean optic lobes that can shed any light on lamina functions. The only recordings from lamina neurons in crustaceans were performed on the crab Leptograpsus (Erber and Sandeman 1976). These recordings showed that crab lamina cells exhibited the same type of hyperpolarizing responses as in insects. Changes in intensity changed the amplitude of the response. It was also found that the resensitization of the lamina cells is more rapid than that of the receptor cells.

The remaining studies on visual interneurons are performed in more central regions, these findings will be discussed at the end of this chapter. The neural connections revealed in the crayfish must thus be discussed mainly in the light of findings on insect lamina neurons.

Intensity and contrast coding, mediated by LMC's in insects most likely is mediated by means of M2 in the crayfish. M2 cells morphologically resemble LMC's in receiving multiple synaptic input from seven photoreceptors each. The numerous parallel synapses of LMC's are also present in M2 cells. It will then remain to be demonstrated if M2 share the properties of LMC's like the neural adaptation, and narrowed angular sensitivity.

There are several substrates for lateral inhibitory pathways. M5 cells can connect M2 cells (directly or via other neurons) in several cartridges. The same is possible for the MP cells (if they are neuronal) and the lamina processes of Tan 1 neurons. The latter possibility requires that Tan 1 neurons form a local circuit system in the lamina and its connection with the medulla serves other functions. Amacrine cells and wide field monopolar neurons (L4) are likely substrates for lateral inhibition in the fly (Strausfeld and Campos-Ortega 1976).

Adaptational pathways are legio and highly complex in the fly (Strausfeld and Campos-Ortega 1976). The data so far

present on the crayfish allow very few conclusions to be drawn. Centrifugal neurons like C1 and perhaps Tan 1 might feed back information from the possibly photoptic R8 cells (ending in the medulla externa). Local circuits for adaptation within the cartridges can also be present (see M3 and M4 below). Finally the catecholamine-neurosecretory system might be involved in neural adaptation.

The stratified monopolar neurons M3 and M4 receive inputs from photoreceptors in each a stratum of the lamina plexiform layer. It has been inferred that the four epl_1 and the three epl_2 receptor terminals respectively of each cartridge represent two classes of photoreceptors. The four in epl_1 would be correlated with photoreceptors having vertical and the three in epl_2 horizontal microvilli (Nässel 1975, 1976a, Stowe et al. 1977, Nässel and Waterman 1977).

If this arrangement is present then M3 would channel information from receptors sensitive to vertical e-vector and M4 from those sensitive to horizontal e-vector. These two possible e-vector channels together with the R8 channel, which possibly is a third e-vector channel have their outputs in the medulla, where neurons sensitive to a rotating e-vector are demonstrated in a crab (Leggett 1976).

The M1 neurons which apparently also channel receptor information are other candidates for e-vector channelling. Whether the M3/M4 system or the M1 system relays this information remains to be demonstrated. In any case one of the systems relays information other than e-vector.

Although the receptors are sensitive to the e-vector, there is no clear demonstration that decapod crustaceans can analyze the e-vector of lineary polarized light (and that the rotating e-vector can be distinguished from variations in light intensity for the crayfish). The use of polarized light in orientation as in insects (von Frisch 1965, van der Glas 1977) remains to be demonstrated for crustaceans.

An interesting feature of M3 and M4 neurons is the small knobs protruding into the receptor terminals near the synaptic sites. These knobs might represent synaptic specialization and thus a kind of reciprocal synapses could be present.

Since the question of the two classes of λ -sensitive cells still is unsolved, it is meaningless to discuss colour coding at the laminar level. Suffice to say that colour information is relayed to deeper optic neuropils (Trevino and Larimer 1970, Woodlock and Goldsmith 1970).

The large Tan 2 neurons have geometries (strictly horizontal and vertical primary processes) making them suggestive as large field motion sensitive units. Such units have not been demonstrated at the laminar level in any arthropod yet. It is clear that the Tan 2 neurons do not directly correspond to the motion sensitive units recorded in the optic nerve of the crayfish (Wiersma and Yamaguchi 1966, 1967, Yamaguchi et al. 1973) since Tan 2 neurons terminate in ME. Tan 2 neurons might, however, relay input on higher order motion sensitive units originating in the medulla.

The neural elements of the crayfish lamina vary in size depending on their location in this neuropil. The central part of the lamina is thicker than the marginal regions and in the central part the largest neurons are found. M2 neurons can be used as an example. The diameter of the M2 lamina axon, the diameter of its dendrites spread and the length of axon possessing lateral lamina dendrites vary. Centrally in the lamina all these values are the highest. This would probably infer a higher number of parallel synapses and thus a stronger summation of receptor input. Accordingly the diameters of the cartridges are the largest in the centre of the lamina (cp. Pandalus; Nässel 1975).

These size variations correspond well to variations of sizes of the corneal facets. These facets are at their

largest in the centre of the retina and are smaller and more irregularly set at the margins.

It could then be assumed, that the central part of the retinal mosaic has units capturing more light and are transferring stronger signals. A kind of fovea mechanism would be present. This fovea in the central part of the retinal mosaic points sideways (slightly anteriorly) for the crayfish.

At present most data on visual interneurons in the crayfish (and other crustaceans) are obtained from the optic nerve. The optic nerve neurons are of higher order (at least 3rd or 4th order), but still some important properties can be discussed in relation to the lamina.

In the optic nerve three main classes of neurons have been established that transmit information from the peripheral visual system to the brain (Wiersma and Yamaguchi 1966, 1967). These classes are sustaining units (tonic "on" cells), dimming units (tonic "off" cells) and motion sensitive units. All units have fairly large visual fields. 14 sustaining units, 4 dimming and 11 movement sensitive units are distinguished in each optic nerve (Wiersma and Yamaguchi 1966).

The individual sustaining and dimming fibres have distinct visual fields and are inhibited by stimulation of adjacent fields (Yamaguchi and Ohtsuka 1973, Arechiga and Yanagisawa 1973). Sustaining units might relay input on optomotor neurons (Yamaguchi pers. comm.) thus mediating optokinetic responses and compensatory eye movements (Horridge and Sandeman 1964). Also motion sensitive units (Sandeman et al. 1975) as well as statocyst neurons (Silvey and Sandeman 1976) are mentioned as part of the optokinetic system.

The motion sensitive neurons are in addition shown to relay input to the so called defense reflex of the crayfish (Glanz 1974). These motion sensitive neurons have highly complex

integrated responses showing habituation phenomena and the circuitry also contains memory functions (Shimozawa et al. 1977).

The inputs on these three groups of neurons are at the earliest in medulla externa, either directly from terminals of lamina-medulla neurons or from third order neurons in the medulla. The input on sustaining units is apparently from neurons representing both e-vector channels and both λ -sensitive channels (Trevino and Larimer 1970, Woodcock and Goldsmith 1970, Yamaguchi 1967). The anatomy of the above mentioned units of the optic nerve remains to be clarified with dye injections. Then the gap between peripheral and more central integration in the optic lobes can be bridged. This also marks how important a study of the second optic neuropil region, the medulla externa, is to appreciate principles in the visual system of the crayfish.

6. COMPARATIVE STUDIES OF CRUSTACEAN OPTIC LAMINAE (Nässel 1975, 1976b, Nässel et al. 1977, and previously unpublished results)

A number of crustacean optic lobes have been investigated. These studies mainly concern decapod crustaceans. The decapods studied are: the lobster Homarus (Hamori and Horridge 1966a, b), the shrimp Pandalus (Nässel 1975), the Norway lobster Nephrops (Nässel 1977), the crayfishes Procambarus (Hafner 1973) and Pacifastacus (Nässel 1977), the crabs Leptograpsus and Scylla (Stowe et al. 1977) and Ocypode (Nässel unpubl.). In addition, another crayfish species, Orconectes, was studied briefly (Nässel unpubl.) and found identical to Procambarus and Pacifastacus. These three crayfish species will therefore not be treated separately.

Some nondecapod crustaceans are also studied with respect to laminar arrangements: the anostracan Artemia (Nässel et al. 1977), and the phyllopod Daphnia (Retzius 1909, Leder 1915,

Wolff and Güldner 1970, Macagno et al. 1973, Nässel et al. 1977). In addition, the mysid Neomysis and the euphausiacean Meganyctiphanes are studied with reduced and selective silver impregnation techniques (Nässel unpubl.).

The laminar arrangements of the different groups are described and specific features that vary within the groups are discussed in the first sections. Then the organizational patterns of the lamina of all the different crustaceans are compared and an attempt is made to clarify basic principles in laminar arrangements.

Decapod crustaceans

There is a surprising uniformity in the neural arrangements of decapod laminae. It is remarkable that decapods with apposition eyes have almost the same types and arrangements of neurons as those with superposition eyes. In this section, only the differences between the previously described crayfish lamina and other decapod laminae will be presented.

All decapods so far investigated have the same types of photoreceptor axons corresponding to RT 1, RT 2 and R8. The axon type designated RTd with its terminal in epl_1 and slender elongation into epl_2 is, however, only found in the crayfish species. The R8 axons are spiny in their lamina portion in crabs (Stowe et al. 1977, Nässel unpubl.), but are smooth in other decapods.

Also the monopolar neurons M1-M4 are almost identical in the studied species. The crayfish, in addition, possesses a fifth type, M5, with a wide field arborization. That no M5 type cells have been demonstrated in other decapods might depend on failure to stain such cells. This might be inferred since this type of cell was impregnated in a low frequency in the crayfish. Stowe et al. (1977) described one monopolar cell type (M5 with their terminology) which has a dendrite

field resembling M3 but a slightly bent axis fibre distally. It is likely that this cell actually is an M3 neuron and not a fifth type. The variations of arborization patterns within individual cell types are very extensive in decapods, but still the very precise synaptic contacts between the different types are retained (Nässel and Waterman 1977, Nässel unpubl.) (cp. Ribi 1976).

Some variation is found in the types and arrangements of tangential neurons in different decapods. Nephrops and Pandalus have basically the same types of tangential neurons (Tan 1 and Tan 2) as the crayfishes, but their lateral lamina processes extend over a much larger field. In contrast to the crayfish Tan 1 neurons in the former two species are clearly bistratified in the lamina, and form two plexa, one in epl_1 , the other in epl_2 . Tan 1 neurons in Pandalus were in a previous investigation (Nässel 1975) only fragmentary stained and were designated Tan:d. With the use of improved rapid-Golgi techniques their complete morphologies were revealed (unpublished) and their identities as Tan 1 determined. Tan 2 neurons of Pandalus were also previously incompletely stained and were designated Tan:p (Nässel 1975).

Crabs possess neurons similar to Tan 1 and Tan 2, designated T 1 and T 3 by Stowe et al. (1977). Also in these species, the Tan 1 neurons are bistratified. A third type of tangential neuron is revealed in Leptograpsus and Scylla (Stowe et al. 1977) and Ocypode (Nässel unpubl.). This neuron type has thin axis fibres and thin perpendicular lamina processes from tangential primary branches below the epl_1 .

In addition, two plexa of thin tangential processes are found in Ocypode, one distal to epl_1 , the other proximal to epl_2 (Nässel unpubl.). Thin fibres penetrate the lamina plexiform layer and connect the two plexa. No fibres connecting these plexa with medulla externa have so far been revealed.

Neurons are described in crabs (Stowe et al. 1977), Pandalus (Nässel 1975) and Pacifastacus (Nässel 1977) that were assumed to be centrifugal by analogy with insect neurons of similar lamina morphologies (Strausfeld 1976). Their morphologies differ between the species. In no case there is evidence that these neurons are indeed centrifugal especially since their cellbodies are not found in the crustaceans.

Multipolar anaxonal cells have only been stained in crayfishes and Nephrops (Nässel 1977). Again this may depend on difficulties to stain such cells and not the lack of them in other decapods. In neither the crayfishes nor Nephrops it is clear if the multipolar cells are neurons or neuroglial cells. In Artemia and Daphnia (see below) cells that most likely are amacrine neurons are present. In Daphnia this appears clear from EM serial sections (Macagno et al. 1975). This might infer that the multipolar cells in decapods are amacrine cells.

At the EM level only the laminae of Homarus (Hamori and Horridge 1966b), Pandalus (Nässel 1976b) and the crayfish (Shivers 1966, Hafner 1973, 1974, Nässel 1976a, Nässel and Waterman 1977) are studied.

Pandalus is the species with the most orderly arrangements of the lamina plexiform layer. The cartridges are distinct, with elements having relatively constant shapes and locations. The Pandalus cartridges are arranged in horizontal rows separated by neuroglial processes and plexa of tangential neuron processes. The other decapods studied have hexagonally arranged cartridges that are less regular in shapes and with disordered arrangements of their constituent profiles.

It is highly probable that the receptor output on the monopolar neurons M1-M4 is the same in the decapods studied. Triad synapse complexes are identified in Homarus (Hamori and Horridge 1966b), Pandalus (Nässel 1976b), the crayfish (Hafner 1974, Nässel 1976a, Nässel and Waterman 1977). Both in

Pandalus and Pacifastacus the central of the three post-synaptic profiles originate from M2 spines. It might be expected that also the neural elements constituting the remaining two profiles of the triads are the same in other decapods as in Pacifastacus (see previous chapter).

The M1-M4 perpendicular pathways, and their basic functional characteristics, thus seem to be of crucial importance in the decapod compound eye. These principles are apparently not affected by apposition contra superposition type of vision.

The remaining connections are only fragmentary known in Pacifastacus and in no other decapods any details are available. There are, however, possibilities for species variations in lateral connections in the lamina and other perpendicular pathways like M5, Tan 1 and Tan 2. Since the types and arrangements of neurons as seen in the light microscope are fairly similar in the different species studied one would, however, not expect any dramatic differences in connectivity patterns.

The major differences in neural arrangements between decapod species appear to be in the medulla externa and deeper neuropils. No detailed accounts are present on crustacean medulla arrangements, but inspections of silver impregnations of the medulla externa in several decapods show organizational differences (Nässel unpubl.). The number of strata and the overall size of the neuropil vary in different species. Also the patterns of aminergic neurons in the medullae differ between different decapods (Elofsson and Klemm 1972).

Mysid and euphausiacean crustaceans

Neomysis integer, a shallow water mysid, was studied with reduced and selective silver techniques (Fig. 3a). The lamina is clearly bistratified, with photoreceptor terminals in two layers epl_1 and epl_2 . Also stratified monopolar neurons, M3 and M4, corresponding to epl_1 and epl_2 are distinguished.

The photoreceptor terminals are enlarged and regular with smooth outlines and the lamina neuropils arranged in a clear periodic pattern.

Neurons of M2 type are also recognized in Golgi preparations, whereas M1 neurons are not. The presence of M1 type neurons is, however, suggestive from reduced silver preparations. There is namely a very regular and distinct inner cellbody layer and thin axons from these cellbodies penetrate the lamina.

Tangential neurons have processes at the distal margin of epl_1 and proximal to epl_2 , where thus plexa of tangential processes are formed. Only in the case of the tangential neurons with processes distal to epl_1 have the axis fibres to medulla externa been resolved. This neuron type appears to be of T-neuron type. In Bodian preparations it can be seen that the tangential processes in the plexus distal to epl_1 occasionally are connected to such in epl_2 by means of thin perpendicular processes. This indicates that some neurons have processes in both layers.

A very characteristic neuron type is resolved that so far is unique to Neomysis. This neuron type connects the medulla externa with the lamina by means of a thin axon. In the lamina plexiform layer, lateral processes are found at two levels. The first level is distally in epl_1 (corresponding to the distally located tangential neuron processes) where varicose and blebbed processes are found. The other is at the midportion of the plexiform layer where several tufts of blebbed processes can be seen. Occasionally these neurons have longer side branches ending with similar tufts in the midportion of the lamina. Thus, these neurons can have specializations in one, two or three cartridges in the lamina. If these neurons are of T-neuron type or are so called centrifugal neurons is not yet clear.

The similarities in the basic organization of mysid and decapod laminae are obvious. The neurons M1-M4 are present in both groups as are receptor terminals in two layers and cartridges. The main differences are: the arrangements of the tangential neurons, the presence of bistratified small field non-monopolar neurons, and the extremely long cellbody fibres of M2-M4.

Meganctiphanes norwegica, a deep sea euphausiid from Norwegian fiords, was also briefly studied with silver impregnation techniques. The Golgi method was less successful and stained only receptor terminals and three types of monopolar neurons.

The receptor terminals have expanded regular trunks either distally, epl_1 , or proximally, epl_2 . M3 and M4 neurons are found corresponding to these lamina strata. epl_1 is slightly thicker than epl_2 (i.e. epl_2 RT's have longer expanded trunks and M4 have lateral processes over a longer axon portion).

M2 neurons are resolved with processes both in epl_1 and epl_2 . All monopolar neurons resolved have very long cellbody fibres, since all lamina cellbodies are situated lateral to the lamina. There is thus no inner cellbody layer and it is uncertain if there are any M1 type neurons.

The lamina neuropil is clearly columnar as seen both in Golgi and Bodian preparations. Reduced silver preparations in addition show two layers of tangential processes in the lamina. These layers are the same as in Neomysis (distal to epl_1 and proximal to epl_2). There are also indications of lateral processes in a thin stratum between epl_1 and epl_2 .

Thus, Neomysis and Meganctiphanes have very similar arrangements in the lamina, but they also have basic similarities to decapods.

Branchiopod crustaceans

These non-malacostracan crustaceans have a much simplified visual system. The retina of the compound eyes in branchiopods is of apposition type with relatively few ommatidia (22 in Daphnia and c. 300 in Artemia). In Artemia and Daphnia which are discussed here, no special pigment cells are present in the retina and the rhabdoms are non-banded and fused (Röhlich and Törö 1965, Güldner and Wolff 1970, Elofsson and Odselius 1975).

The optic lobes are small and reduced in number. Only a lamina and one medulla neuropil (medulla externa) are present in each eyestalk of Artemia (Hanström 1924). The remaining optic neuropils are withdrawn into the protocerebrum and cannot be distinguished. The same is true in Daphnia except that the two eyestalks are fused to one cyclopic eye, containing two fused retinae and laminae and two medullae externae still exist (Retzius 1906, Elofsson and Dahl 1970). There is no chiasma formed by the fibres connecting the lamina with the medulla in either of these animals as can be seen in malacostracans (Elofsson and Dahl 1970).

Artemia. The lamina of Artemia comprises five classes of neurons: receptor axons, monopolar neurons, tangential T-neurons, centrifugal and amacrine neurons.

The receptor axons are of three types (RT1, RT2 and R1). RT1's end in the distal part of the lamina (epl_1) and RT2's in the proximal (epl_2). Both types have expanded terminals with long thin lateral processes. RL axons end in medulla externa, but have some lateral processes in the lamina plexiform layer.

Monopolar neurons of four types are distinguished (M1-M4). M1 neurons have few or no spines in the lamina, whereas M2's have radially arranged processes throughout the lamina plexiform layer depth.

M3 and M4 neurons are stratified. M3 have lateral processes in epl_1 only and M4 in epl_2 . There is an extensive variation in branching patterns among the monopolar neuron types sometimes making it difficult to classify individual neurons.

Tangential T-neurons (Tan) have tangentially arranged long processes both in epl_1 and epl_2 and an axon connecting the lamina portion directly with protocerebrum. The cellbodies are situated in the medulla cellbody layer (lateral to the medulla) and are connected to the axons with cellbody fibres in a T-neuron fashion.

Centrifugal neurons have their cellbodies in the medulla cellbody layer and axons passing through the medulla to the lamina where long lateral processes are found. In the medulla the centrifugal neurons have lateral processes in a distinct stratum close to the medulla cellbody layer.

Amacrine neurons have their cellbodies distal to the lamina plexiform layer and processes profusely branching in the plexiform layer. These neurons can be either monopolar or multipolar but never have any axons to the medulla.

With EM it is possible to disclose a periodic organization of the Artemia lamina distally. Pseudocartridges with 6 photoreceptor axons and 4-5 other axons can be identified. The axons of the pseudocartridges enlarge and become heavily branched upon entering the lamina plexiform layer. Distinct cartridges cannot be resolved, but there is a periodisism of neural elements. Most likely, functional units corresponding to cartridges are present, though the lateral interaction with neighbouring units is very extensive in Artemia.

The photoreceptor-second order neuron synapses are of dyad synapse type. An evaginating membrane area of the RT containing a dense bar and clear vesicles face two postsynaptic profiles (cp. decapods with three postsynaptic profiles).

The photoreceptor terminals form specialized contacts with each other. These contacts are characterized by a finger-like protrusion from one terminal into another. Clear vesicles are clustered on one or both sides of the membrane contacts. It is, however, not clear if these contacts are true synapses.

Another feature of interest is the presence of nervous profiles with small dense cored vesicles. These profiles form part of a tangential network of fibres in the plexiform layer. No profiles with large dense vesicles are found as seen in decapods (Shivers 1966, Nässel 1976a, Elofsson et al. 1977) and Daphnia (Wolff and Guldner 1970). Aramant and Elofsson (1976) demonstrated presence of catecholaminergic neurons in the Artemia lamina, which possibly could be related to the profiles with small dense cored vesicles.

Daphnia. Daphnia comprises the same classes of lamina neurons as Artemia. There are, however, some differences in details. The photoreceptor axons are of three types (RT1-RT3) ending in three strata, but none of these end in the medulla. Long photoreceptor axons ending in the medulla have never been resolved in Golgi or vital staining techniques (Retzius 1906, Leder 1915). Such axons were, however, suggested by Macagno et al. (1975), who used computer aided reconstructions from serial EM sections.

The RT's have enlarged terminals in the lamina plexiform layer: RT1 distally in the plexiform layer and RT3 proximally; RT2 axons have longer terminal trunks in the midpart of the lamina plexiform layer. All types have long thin lateral processes. The variation of the receptor terminal morphology and branching patterns is extensive as also described by Macagno et al. (1973).

Only two types of monopolar neurons are recognized. One type is bistratified with processes corresponding to RT1 and RT3. The other type has processes in a broad portion of the mid-stratum of the lamina more or less corresponding to RT2's.

Retzius (1906) and Leder (1915) showed two types of connections between the lamina and the brain (protocerebrum) represented by two types of tangential neurons. These have also been identified by Nässel et al. (1977). One type directly connects the lamina and protocerebrum, the other connects both the lamina and the medulla with protocerebrum. The cellbodies of these types of neurons are situated in the medulla cellbody layer.

Both centrifugal and amacrine lamina neurons are also recognized in Daphnia. In Daphnia pseudocartridges can be resolved containing 8 photoreceptor axons and five other axons. In the lamina plexiform layer, however, no clear columnar or periodic pattern can be resolved. The extensive branching of photoreceptor and second order neurons obviously conceal such a pattern. Most likely Daphnia as well as Artemia have periodic functional units of photoreceptor axons and second order neurons even though these are not separated as true cartridges.

In Daphnia no dyad synapse complexes are seen but instead 1:1 synapses of 2-3 types can be seen (Wolff and Güldner 1970, Nässel et al. 1977). Macagno et al. (1973) revealed that the photoreceptor-second order neuron synapses were of this 1:1 type.

In Daphnia both fibres with small dense cored and such with large dense vesicles are distinguished (Wolff and Güldner 1970).

The differences in neural organization between Daphnia and Artemia are in short:

1. Possibly the layering of RT's in Daphnia differs from the bistratified pattern in Artemia.
2. Daphnia monopolar neurons of only two types are found so far (cp. Artemia with 4 types).
3. In Daphnia no long receptor axons are so far found.
4. No dyad synapses are found in Daphnia.
5. Neurosecretory fibres occurring in Daphnia are absent in Artemia.

6. No wide field centrifugal neurons are resolved in Daphnia.
7. The presence of tangential neurons connecting the lamina and the medulla with protocerebrum in Daphnia.

Features characteristic for branchiopods are:

1. The RT's with long lateral processes.
2. The absence of clear columnar organization of the lamina.
3. Neurons directly connecting the lamina with protocerebrum.

General comparisons of neural arrangements in crustacean laminae

The decapod, mysid and euphausiacean crustaceans investigated so far all have the same basic organization of the lamina. This could to some extent be a reflection of possessing rhabdoms of the same type. The species studied here all have rhabdoms with layers of orthogonally arranged microvilli, i.e. two classes of photoreceptor cells. The two layers of receptor terminals might be correlated with these classes of cells.

Also the lamina neural arrangements are very similar and either reflect phylogenetic similarity or adaptational convergence to form substrates for basic neural principles at the laminar level. In any case, the lamina apparently has a relatively constant circuitry integrated into visual systems with different retinal and medullary complexities. The different laminae would receive information of different complexities (due to number of retinal facets, screening and photo pigment types and dioptric properties). The signals are then integrated and channelled in the periodic lamina mosaic basically in the same manner. The lamina signals are finally integrated in higher neuropils of varying complexities in the different animals and the visual information thus available for the animals will be of varying qualities.

The branchiopods studied have fused unbanded rhabdoms and are phylogenetically well separated from the malacostracans. Thus, it is difficult to relate differences in laminar arrangements to retinal structure only.

There are some similarities between malacostracan and branchiopod laminae studied. Monopolar neurons like M2 are present in all species, that apparently summates input from all photoreceptor axons of a laminar synaptic compartment. Stratified monopolar neurons receiving input from a fraction of these receptors are also present (this is not clear in Daphnia yet). Apparently summation and channelling of receptor signals is performed in all studied crustacean laminae.

The branchiopods then appear to have a lesser degree of processing of receptor signals as indicated by direct connections between the lamina and the protocerebrum, the small medulla externa and lack of medulla interna.

As a comparison, it can be mentioned that insect laminae studied show a much more extensive variation in neural arrangements (Strausfeld 1976) though also here some basic patterns can be traced. Two of the patterns so far resolved that are in common for insects and crustaceans are the presence of more or less separated functional laminar units, cartridges, and the large monopolar neurons receiving inputs from all receptors of one such unit.

Other more general patterns shared are the presence of monopolar, tangential, centrifugal and occasionally amacrine neurons. The comparison can in such general terms be extended to cephalopods and vertebrates whose retinae possess neurons of the same basic patterns (Cajal and Sanchez 1915, Cajal 1917, Zawarzin 1950, Strausfeld and Campos-Ortega 1977; see also Weiler and Zettler 1976, Laughlin 1976). And thus it most likely is as the above authors suggest that some basic neural principles underlying vision are in common for arthropods, cephalopods and vertebrates.

GENERAL SUMMARY

The first chapter describes the retinal morphology in the crayfish. Some of the functional properties (spectral sensitivity, polarization sensitivity, and range of absolute sensitivity) are discussed in the light of recent investigations.

The second chapter attempts to reveal the projectional patterns of photoreceptor axons between the retina and the first optic neuropil, the lamina. Previously published results (e.g. Nässel 1976a) are put in relation to more detailed recent studies on other crustaceans (Stowe 1977). The presence of a direct (the axons from one ommatidium projects to one cartridge) contra a divergent projection (the axons project to several cartridges) is discussed.

The next two chapters give descriptions of types and arrangements of crayfish laminar neurons. 3 types of receptor axons, 5 types of monopolar, 3 types of tangential neurons, 2 types of multipolar cells and 1 type of centrifugal neuron are found and their relations are clarified. One of the tangential neuron types is catecholaminergic and its possible function is discussed.

Chapter 5 describes the connectivity patterns of four types of monopolar neurons as revealed with the Golgi-EM method. The possible functional properties of the lamina are discussed (properties such as visual information channelling, summation of receptor signals, narrowing of ommatidial visual fields, neural adaptation and movement detection).

Finally, the sixth chapter gives a comparison of laminar arrangements in several decapod crustaceans and the mysid Neomysis, the euphausiacean Meganyctiphanes, and the branchiopods Daphnia and Artemia. An attempt is made to reveal neural principles in crustacean laminae and relate these to findings on insect visual systems.

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VON ROSENHOF (1755)

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FIGURE LEGENDS

- Fig. 1a Cartridge in epl_1 with Golgi-stained M1 neuron. Arrows point at silver chromate filled profiles of M1 neuron contacting only two of the epl_1 RT's. Magnification: x 7 600
- Fig. 1b Triad complex in epl_1 formed by spines of M1, M2 and M3 monopolar neurons. These profiles are post-synaptic to an RT filled with clear vesicles. Note the dense arrow-like presynaptic bar. inv = neural spine invaginating into RT. Magnification: x 93 300
- Fig. 1c Triad complex in epl_1 with silver chromate filled M1 profile. RT = receptor terminal. Magnification: x 38 300
- Fig. 2 Schematic drawings of cartridges. M1-M4 = monopolar neurons. RT = receptor terminals.
- Fig. 2a Exploded view of cartridge to show the locations and some of the relations of the receptor terminals and monopolar neurons. The spines of M1a and M1b are not drawn.
- Fig. 2b Schematic representation of connections between monopolar neurons and receptor terminals. Arrow heads indicate synaptic triad complexes.
- Fig. 3 Lamina neurons of Nemysis integer. M2-M4 = monopolar neurons (cellbodies are not shown). Centrifugals with processes in one (C1) two (C2) and three (C3) cartridges are shown. Tan = tangential neuron with processes distal to epl_1 . RT = receptor terminals in two strata (epl_1 and epl_2).

Fig. 4 Lamina neurons of Meganyctiphanes norwegica.
M2-M4 = monopolar neurons (cellbodies are not
drawn) note the long cellbody fibres.
RT = receptor terminals in two layers (epl₁ and
epl₂).

